University of Alberta

Steroids, Dietary Lipids, Resection and Intestinal Adaptation

by

Aducio Leonel Thiesen Junior ©

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fulfilment of the requirements for the degree of Doctor of Philosophy

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Steroids, Dietary Lipids, Resection and Intestinal Adaptation** in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Medical Sciences–Medicine .

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ABSTRACT

Intestinal adaptation is the ability of the intestine to respond to changes in the environment. Intestinal resection is the classical model of adaptation. Orally administered steroids enhance the sugar absorptive functions of the intestine, but their effect on lipid uptake is unknown. Modifications in dietary lipids alter the nutrient transport properties of the intestine, but it is unknown if there is an interaction among dietary lipids, steroids and intestinal resection on the intestinal uptake of sugars and lipids. Accordingly, this study was undertaken to assess the influence of prednisone, dexamethasone, and the locally acting steroid budesonide in male Sprague Dawley rats fed chow or either a saturated fatty acid diet (SFA) or a polyunsaturated fatty acid diet (PUFA), and rats undergone intestinal transection or a 50 % intestinal resection. Giving budesonide or prednisone up-regulates D-fructose uptake, and enhances the uptake of some lipids. Feeding PUFA prevents the prednisone- or budesonide-associated enhanced uptake of fructose in rats fed SFA, whereas feeding SFA diet increases the effect of these steroids on the absorption of lipids. In resected animals, budesonide increased the jejunal uptake of D-glucose and the ileal uptake of D-fructose, and the uptake of lipids was unchanged. Dietary lipids prevented the effect of budesonide on the uptake of sugars and did not change the uptake of lipids. Transporter abundance and expression of SGLT1, GLUT5, GLUT2, Na^+/K^+ ATPase, L-FABP and ILBP did not explain the phenotypic alterations in the absorptive function, but immunohistochemistry for SGLT1 and GLUT5 was able to explain the enhancement of glucose and fructose uptake in animals fed SFA. The signals studied ODC, proglucagon, ERG and cytokines did not explain the adaptive responses in the three models studies. The adaptive effect of steroids on intestinal is influenced by

dietary lipids and modified by intestinal resection by a mechanism other than differences in food intake, body weight gain, villus height, transporter expression and abundance. Steroids, when given in doses which are effective clinically, change the intestinal absorption of sugars and lipids by a process which is modified by the dietary content of lipids and intestinal resection.

DEDICATION

Carinhosamente dedico o fruto do meu esforco aos meus pais, Aducio Leonel Thiesen e Lucia Maria de Souza Thiesen.

Gostaria tambem de lembrar todos os amigos que estiveram presentes durante o progresso desta tese.

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LIST OF ABBREVIATIONS

5-ASA	5-aminosalicylic acid
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
AP1	activating protein 1
BBM	brush border membrane
BLM	basolateral membrane
BrdU	bomodeoxyuridine
BUD	budesonide
CBG	corticosteroid-binding-globulin
CDAI	Crohn's disease activity index
CIR	controlled ileal release
DEAE	diethylaminoethyl
EGF	epidermal growth factor
EMM	enterocyte microsomal membrane
ERG	early response genes
ERK c	extracellular signal regulated kinase
FFA	free fatty acids
GH	growth hormone
GLP2	glucagon like peptide 2
GTP	guanosine 5'-triphosphate
Hc	hydrocortisone
HNF-4	hepatocyte nuclear factor 4

IGF	insulin-like growth factor
IGF BP	insulin-like growth factor binding proteins
IL-6	interleukin-6
JAK	janus-kinase
Km	apparent affinity constant
LDL	low density lipoprotein
MIF	migration inhibition factor
MPred	methyl prednisolone
MTV-CAT	mouse mammary tumor
NADH	nicotinamide adenine dinucleotide
NfκB	nuclear factor kappa B
NT	neurotensin
ODC	ornithine decarboxylase
ODC	ornithine decarboxylase
Pd	passive permeability coefficient
PGP	P-glycoprotein
PKC	protein kinase C
PL	placebo
PPAR	peroxisome proliferator-activated receptor
PRED	prednisolone
PUFA	polyunsaturated fatty acid
SBS	short bowel syndrome
SC	subcutaneous

SFA	saturated fatty acid
SI	sucrase-isomaltase
SREBP1c	steroid response element binding protein
TRF	thyrotropin-releasing factor
TSH	thyroid stimulating hormone
Vmax	maximal transport rate

A) INTRODUCTION

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis [Brattsand, 1990; Brignola et al., 1992; Brignola et al., 1994; Danielson and Prytz, 1994; Lofberg et al., 1993; Nyman-Pantelidis et al., 1994; Tarpila et al., 1994; Greenberg et al., 1996; Palmen et al., 1998; Thiesen and Thomson, 1996; Sandborn and Faubion, 2000; Lundin et al., 2001] . However, systemic steroids may be associated with numerous and potentially serious adverse effects [Brattsand, 1990; Girdwood and Petrie, 1987; Haynes and Murad, 1985; Van Ierssel et al., 1997; Palmen et al., 1998] . Even topical treatment with steroids using retention enemas or foams depresses plasma cortisol concentrations [Cann and Holdsworth, 1987; Reshef et al., 1992; Caesar et al., 1997]. For this reason, non-systemic steroids have been developed. The non-systemic steroid budesonide has high topical activity, low systemic bioavailability, and rapid first pass metabolism [Brattsand, 1990; Palmen et al., 1998; Lundin et al., 2001]. Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease [Danielson et al., 1992; Greenberg, 1994a; Greenberg, 1994b; Rutgeerts et al., 1994; Palmen et al., 1998; Cortot et al., 2000a; Cortot et al., 2000b; Cortot et al., 2000c].

Intestinal adaptation is a process that occurs in response to physiological or pathological processes such as intestinal resection, aging, diabetes, abdominal radiation, fasting and malnutrition, chronic alcohol intake, and feeding diets of varying lipid, protein or carbohydrate composition [Thomson et al., 1990; Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Ferraris and Carey, 2001]. Thus, there are different morphological and physiological characteristics of the intestine according to the specific nutrient composition of the diet [Diamond and Karasov, 1984; Thomson and Rajotte, 1983a; Thomson and Rajotte 1983b; Thomson, 1982; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Ferraris and Carey, 2001]. In adult rats, the intestinal uptake of sugars and lipids is enhanced by feeding an isoenergetic semisynthetic diet enriched with saturated as compared with polyunsaturated fatty acids [Thomson et al., 1986; Thiesen et al., 1996; Thiesen et al, 2000].

Steroids induce precocious development of the intestinal brush border membrane (BBM) enzymes, and facilitate the induction of several BBM enzymes by dietary carbohydrate [Deren et al., 1967; Lebenthal et al., 1972; Murphy, 1982; Perin and Thomson, 1998; Thiesen et al., 2000]. Steroids given by mouth enhance glucose absorption by adult animals [Batt and Peters, 1976; Batt and Peters, 1975; Batt and Scott, 1982; Thiesen et al., 2000], but dexamethasone given subcutaneously blunts the expected morphological adaptive response following intestinal resection: mucosal growth and mucosal hyperplasia [Park et al, 1994]. The adaptive effect of locally or systemically acting steroids on intestinal nutrient transport associated with changes in dietary lipids has not been described.

Budesonide enhances the immunosuppressive effects of cyclosporin A and prolongs small bowel allograft survival without affecting normal adrenocorticotrophic hormone (ACTH) release [Freeman et al., 1996; Ozcay et al., 1997]. Therefore, budesonide may be used as a immunosuppressive agent in intestinal transplantations.

Accordingly, this study was conducted to assess 1) the influence of budesonide and prednisone (PRED), in doses equivalent to those used in clinical practice, on the intestinal uptake of sugars and lipids in young growing male rats; 2) the influence of feeding isocaloric semisynthetic saturated or polyunsaturated diets on the intestinal uptake of sugars and lipids in young rats receiving four weeks oral gavage of either the systemical active glucocorticosteroid PRED, or the topically active steroid budesonide (BUD); 3) the influence of intestinal resection on the intestinal absorption of sugars and lipids in adult male rats receiving two weeks of steroids and feeding isocaloric semisynthetic saturated or polyunsaturated diets; 4) the protein and mRNA expression of the transporters responsible for sugar and lipid uptake; and 5) the possible signals involved in this response such as ornithine decarboxylase (ODC), proglucagon, early response genes (ERG) and cytokines.

In order to lay the foundation for these studies, I will present a review covering the absorption of sugars and lipids followed by a consideration of the concepts, mechanisms and examples of intestinal adaptation including these achieved by modifications of dietary lipids, and finally a detailed description of glucocorticosteroids and their effect on the intestine.

B) LITERATURE REVIEW

1) Normal Mechanisms

The mechanisms of nutrient uptake are remarkably efficient, allowing less than 5 % of ingested carbohydrate, fat and protein to be excreted in the stool of adults. Specific mechanisms and transporters have been related to the uptake of sugars, lipids and proteins.

1.1) Sugar Uptake

Since glucose plays a important role in the metabolism of most cells, the transport of D-glucose across plasma membrane is a crucial cellular event. It has been determined by Crane that the Na^+-gradient across the intestinal epithelial cell provides the driving force responsible for the glucose transport through the brush border membrane of the enterocyte [Crane, 1962; Crane et al., 1965; Riklis and Quastel, 1958]. The Na^+-gradient is maintained by the action of the Na^+K^+-ATPase which is restricted to the basolateral membrane [Hirayama et al., 1992]. The Na^+-dependent glucose transporter (SGLT1) mediates the brush border membrane (BBM) Na^+/glucose cotransport [Vehyl et al., 1993; Vehyl et al., 1992; Weber et al., 1991].

The transporter responsible for the exit of glucose through the basolateral membrane (BLM) of the enterocyte is the Na^+-independent sugar transporter (GLUT2). This passage across the BLM occurs by facilitative Na^+-independent diffusion [Caspary and Crane, 1968].

Three kinetic parameters: the maximal transport rate (Vmax), the Michaelis constant (Km) and the passive permeability coefficient (Pd) have been used to characterize the properties of the intestinal glucose transport [Crane et al., 1965; Thorens et al., 1990; Fine et al., 1993]. Correction for the effective resistance of the intestinal unstirred water layer (UWL) has been demonstrated to be necessary when estimating the kinetic parameters of sugar uptake. For instance, without correction for the effective resistance of the intestinal UWL, the value of Km is overestimated whereas Pd is underestimated. Although the Vmax is not altered by UWL, the uptake of higher concentrations of hexoses involves both an active and a passive component and failure to correct for the UWL effects on the passive component may lead to errors in the estimation of the value of Vmax [Steven et al., 1990; Takata et al., 1992; Thomson and Dietschy, 1977; Thomson and Dietschy, 1980; Thomson and Wild, 1997a; Thomson and Wild, 1997b].

1.1.a) SGLT1

The Na^+-dependent glucose transporter was first cloned by Wright and his colleagues [Hediger et al., 1987]. In humans, the SGLT1 gene has been mapped to the distal arm of chromosome 22 [Hediger et al., 1989], and encodes polypeptides of 664 amino acids. The functional SGLT1 transporter is about 290 kb, and is thought to be a homotetramer [Smith et al., 1992].

Two potential sites for glycosylation at positions 248 and 306 have been identified. The post-translational glycosylation adds about 15 kd to the apparent mass of SGLT1 [Hediger et al., 1987]. SGLT1 does not have the Arg-X-G-ly-Arg-Arg sequence characteristic of all other sugar transporters [Hediger et al., 1987; Loo et al., 1992]. A potential site for protein kinase A has been recognized in SGLT1 and the binding sites of SGLT1 to glucose were increased by phosphorylation of the sites of SGLT1 in BBM. These findings indicate that the regulation of phosphorylation of SGLT1 leads to an alteration of its function, and results in the activation of glucose transport in the rat small intestine [Ishikawa et al., 1997; Scholtka et al., 1999]. By the same token, potential roles of PKC and MAP kinase intracellular signalling pathways in the regulation of SGLT1 have been proposed, but the details of this mechanism are unknown [Vayro and Silverman, 1999; Kellet and Helliwell, 2000; Kellet, 2001]. However, the use of phorbol 12-myristate 13-acetate (PMA) and bisindolylmaleimide, an agonist and inhibitor of PKC respectively, characterizes the reduction of Vmax for SGLT1 when PKC is activated [Vayro and Silverman, 1999].

Another factor involved in the regulation of SGLT1 involves epidermal growth factor. EGF increases the expression of SGLT1 by a mechanism that depends on actin polymerization, suggesting regulation of EGF at the internal trafficking of SGLT1 from the ER to the BBM [Chung et al., 1999]. In rabbits after a 70% small bowel resection, EGF improves gut function by normalizing SGLT1 distribution along the villus-crypt unit and increasing apoptosis, without changing the expression or abundance of SGLT1 [Chung et al., 2001]. Still in rabbits, but a 60% intestinal resection, EGF enhances glucose uptake in the remnant intestine increasing the absorptive surface area [Hardin et al., 1999].

SGLT1 binds two Na^+ for each glucose molecule. The binding of Na^+/Na^+ markedly reduces the Km for glucose from about 100 mM to 0.1 mM. In the presence of a Na^+-gradient, the rate-limiting step in glucose uptake is the return of SGLT1 from the cytoplasmic to the luminal side of the BBM. When the glucose concentration in the cytoplasmic gets too high, glucose stops coming from the SGLT1 and the Vmax falls [Crane et al., 1965; Murer and Hopfer, 1974; Boyd et al., 1975]. An interaction between the activities of SGLT1 and GLUT2 must occur in order for there to be an increase of cytoplasmic glucose concentration [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Hirayama et al., 1997]. Recent work suggests that this interaction between SGLT1 and GLUT2 is complex involving possible mobilization of GLUT2 towards the BBM through a cascade of signals initiated by SGLT1. Among theses signals evidence points to PKC IIβ [Kellet, 2001].

Structural analysis of SGLT1 predicts that this transporter spans the BBM 14 times [Turk et al., 1996; Eskandari et al., 1998; Bissonnette et al., 1999]. An aspartic acid is conserved at the interface between the first transmembrane segment and the NH2-terminal. Mutations such as substitution from Asp28 to Asn28 impair sugar uptake resulting in the rare child with glucose-galactose malabsorption [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Martin et al., 1997; Martin et al., 1996; Desjeux and Wright, 1993; Turk et al., 1991].

Since the glucose transport by SGLT1 is only observed from the luminal to the intracellular side of the BBM, and since phlorizin inhibits SGLT1 only from the luminal side, it is concluded that SGLT1 is an asymmetric protein [Karasov and Diamond, 1983; Koepsell et al., 1988; Lazaridis et al., 1997; Sarker et al., 1997; Suzuki et al., 1996]. Cyclosporin binding sites are present on the extracellular side of SGLT1, and in fact cyclosporin will decrease the Vmax for glucose uptake [Sigalet et al., 1996].

The use of monoclonal antibodies against the renal Na^+-dependent glucose transporter that react with intestinal SGLT1 allowed the detection of higher antigenic sites in the jejunum, intermediate in ileum and lower in the duodenum. However, no differences in SGLT1 expression along the length of the villus were realized [Gould and

Holman, 1993; Koepsell et al., 1988; Freeman et al., 1993; Hopfer, 1987; Burant et al., 1994]. Using polyclonal antibodies against rabbit intestinal BBM SGLT1, crypt cells show no staining and the onset of SGLT1 appears to start at the crypt-villus junction [Hwang et al., 1991]. The amount of mRNA for SGLT1 also increases towards the mid-portion of the villus, then falls slightly towards the villous tip [Freeman et al., 1993; Li et al., 1998]. Periodicity in glucose absorption has been demonstrated to correlate with mRNA expression of SGLT1, suggesting the existence of acute regulatory mechanisms in the expression of SGLT1 and perhaps a response of this transporter to hormonal factors [Tavakkolizadeh et al., 2001].

The functional SGLT1 consists of two subunits: a catalytic one and a regulatory one, both having a molecular weight of about 70 kd [Vehyl et al., 1992; Poppe et al., 1997]. It is speculated that dietary and hormonal manipulations might affect the expression of SGLT1, modulating the transcription and post-transcription processing of the regulatory subunit of SGLT1 [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Dong et al., 1997]. Protein kinase A and protein kinase C regulation of SGLT1 has also been proposed [Wright et al., 1997; Ishikawa et al., 1997]. Activation of PKC as mentioned before decreases the activity of SGLT1 whereas activation of PKA increases or potentiates glucose uptake [Vayro and Silverman, 1999; Ishikawa et al., 1997].

1.1.b) SGLT2

Low stringency screening with high affinity to SGLT1 has been used to isolate a 2271-nucleotide base pair cDNA from human kidney [Mackenzie et al., 1996]. This clone encodes SGLT2 which presents 59% homologous at the amino acid level to SGLT1 and is present in trivial levels in the intestine [Barfuss and Sachfter, 1981].

1.1.c) GLUT2

The sugar transporter restricted to the BLM facilitates the transport of glucose, galactose, mannose and fructose is named GLUT2 [Thorens et al., 1988; Thorens et al., 1990; Burant and Bell, 1992; Cheeseman, 1993; Maenz and Cheeseman, 1987]. It has 55 % homology with the amino acid sequence of GLUT1, a fact that allowed the cloning of GLUT2 using GLUT1 cDNA probe for screening rat and human liver cDNA libraries under conditions of low stringency [Thorens et al., 1988]. GLUT2 has an apparent molecular weight of 61 kd, and GLUT2 is expressed along the villus, being absent from the crypts. Like all GLUT isoforms, GLUT2 is predicted to have 12 transmembrane domains, with the transmembrane segments 7-8 forming the substrate (glucose or fructose) binding domain, and the transmembrane segments 9-12 modulating the affinity between substrate and transporter [Wu et al., 1998].

Potential sites for cAMP regulation in the promoter of the GLUT2 gene have been described. In fact, mutants not expressing the -312/+49 bp region of the GLUT2 promoter do not show the characteristisc inhibition of cAMP in GLUT2 mRNA levels [Rencurel et al., 1997]. Other possible regulatory mechanisms are possibly involved in the regulation of GLUT2 such as SGLT1 and PKC IIβ [Kellet, 2001].

GLUT2 may also be located in the BBM [Helliwell et al., 2000a; Helliwell et al., 2000b; Kellet and Helliwell, 2000; Kellet, 2001]. A new model of sugar absorption has been proposed (Figure 2) in which GLUT2 is the main regulator of sugar absorption, transporting glucose and fructose through the BBM in the cell, as well as through the

BLM into the blood. GLUT2 is only present in the BBM under particular conditions such as an increase of substrate for SGLT1. In that way, SGLT1 would be the initiator factor that detects the signals and leads to trafficking of GLUT2 from the BLM or an intracellular pool to the BBM. PKC could be one of the intracellular signals responsible for this detection-trafficking event [Kellet, 2001].

1.1.d) GLUT5

Fructose is absorbed by facilitated diffusion, a process that is mediated by a member of facilitative sugar carriers named GLUT5 or Na^+-independent fructose transporter [Burant et al, 1992a, Burant et al., 1992b; Crouzoulon and Korieh, 1991; Davidson et al., 1992; Rand et al., 1993a; Rand et al., 1993b; Shu et al., 1997; Wasserman et al., 1996]. It presents a Km for fructose uptake of 6 mM, and it has an apparent molecular weight of about 46 kd. GLUT5 is confined to the BBM, and is maximally expressed at the tip of the villus, contrasting with the maximum level of GLUT5 mRNA in the mid-villus region [Davidson et al., 1992; Rand et al., 1993a; Rand et al., 1993b; Shu et al., 1997; Wasserman et al., 1996].

Possible regulation of GLUT5 involving cAMP has been suggested, since treatment of Caco-2 cells with forskolin, an activator of cAMP, increases the level of GLUT5 mRNA [Brot-Larohec et al., 1992]. Analogs of forskolin that do not affect adenylate cyclase activity do not change the expression of GLUT5, reinforcing the possible influence of cAMP on GLUT5 regulation.

Periodicity or diurnal variation in the mRNA expression of GLUT5, as seen with SGLT1, has been demonstrated with the corresponding peak before evenings [Castello et al., 1995; Corpe et al., 1998]. Protein synthesis inhibitors like cycloheximide do affect

GLUT5 mRNA expression in the morning, but do not change the enhanced expression in the evening. This suggests complex regulatory mechanisms in the mRNA expression of GLUT5, or at least the existence of more than one regulatory component [Corpe et al., 1998].

During weaning, when there is a shift from a diet high in fat and low in carbohydrates to one which is low in fat and rich in carbohydrates, an enhanced uptake of fructose is observed. This weaning-associated rise in fructose uptake is accompanied by increased levels of GLUT5 mRNA [Shu et al., 1997]. It is tempting to speculate possible correlation of GLUT5 activity and expression with corticosterone levels. However adrenalectomized rats still express the characteristic up-regulation of GLUT5 at weaning discarding the corticosterone role in this ontogenic process [Monteiro and Ferraris,1997].

Figure1. Classical Model of Sugar Uptake [Keelan e al., 1998]

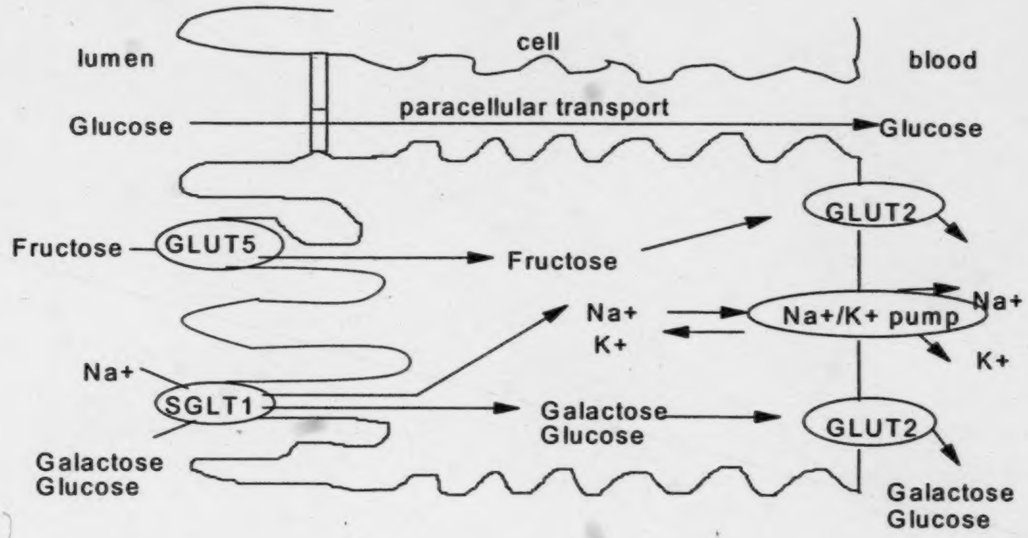
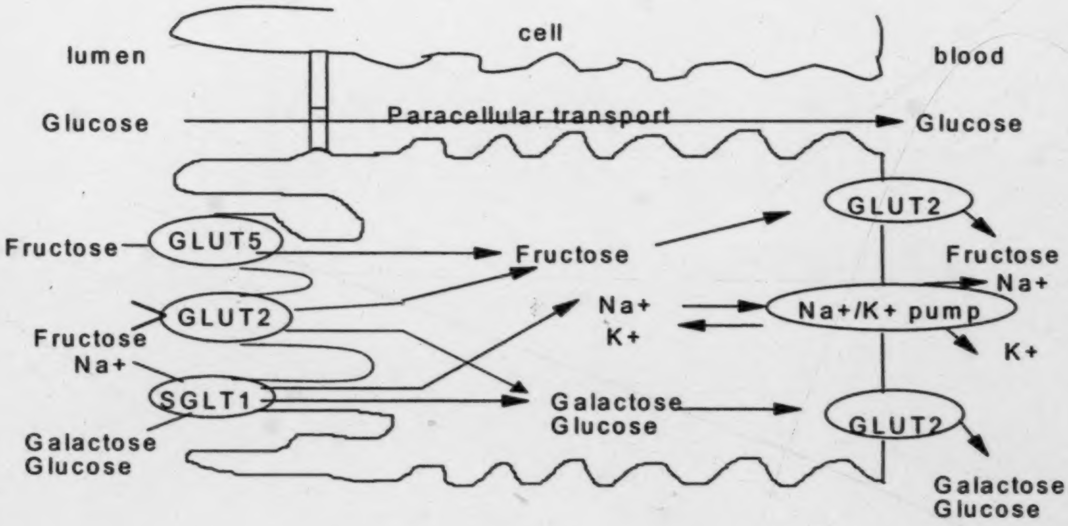

lumen
cell
blood
Glucose
paracellular transport
Glucose
GLUT2
Fructose
GLUT5
Fructose
Na+
K+
Na+/K+ pump
Na+
K+
Na+
SGLT1
Galactose
Glucose
GLUT2
Galactose
Glucose
Galactose
Glucose

Figure 2. Proposed New Model of Sugar Uptake [Kellet, 2001]


lumen
cell
blood
Glucose
Paracellular transport
Glucose
GLUT2
Fructose
GLUT5
Fructose
Fructose
GLUT2
Fructose
Na+
K+
Na+/K+ pump
Na+
K+
Na+
SGLT1
Galactose
Glucose
GLUT2
Galactose
Glucose
Galactose
Glucose

1.2) Lipid Uptake

Most lipids are absorbed in the proximal intestine, more specifically at the upper portion of the villus tip [Borgstrom et al., 1957; Haglund et al., 1973; Ladman et al., 1963; Fingerote et al., 1994]. The products of lipid digestion (free fatty acids, monoacylglycerols, lysophosphatidylcholine and free cholesterol) must initially diffuse through the UWL and then across the BBM [Clandinin and Thomson, 2000; Besnard and Niot, 2000]. These products of lipid digestion are solubilized in mixed micelles composed of cholesterol, bile acids (mainly taurine or glycine conjugates of cholic and chenodeoxycholic acids) and phospholipids (phosphatidylcholine). Once in the cytoplasm, the lipids are taken up by the cytoplasmic fatty acid binding proteins, are incorporated into lipoproteins, and then leave the enterocyte either in lymph, or in portal blood [Chow and Hollander, 1979; Stremmel, 1988; Schoeller et al., 1995a; Schoeller et al., 1995b; Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Hauser et al., 1998; Clandinin and Thomson, 2000; Besnard and Niot, 2000].

1.2.a) Passive Lipid Uptake

Classically lipid absorption has been defined as an energy-independent, passive diffusion process down a concentration gradient. Short- and medium-chain fatty acids are not influenced by the UWL, while passive long-chain fatty acid uptake is rate-limited by passage through the UWL [Proulx et al., 1984a; Proulx et al., 1984b; Proulx et al., 1984c; Westegard and Dietschy, 1974].

Three models of passive lipid uptake have been proposed [Thomson and Dietschy, 1981]:

- The entire mixed micelle is absorbed by the BBM. No experimental evidence for this model has been published [Wilson and Dietschy, 1972]
- The micelle collides with the BBM allowing lipids to be taken up directly. The collision model has been proposed for the cholesterol uptake [Proulx et al., 1984c; Burdick et al, 1994].
- The micelle dissociates in the aqueous compartment of the UWL, releasing the lipids that then are taken up by the BBM. This model has been proposed for fatty acid uptake as well as cholesterol uptake [Westegard and Dietschy, 1976; Chijiiwa and Linscheer, 1987].

Modifications of this last model have been proposed. The dissociation of lipids from the mixed micelle would be under the influence of the acidic microclimate of the UWL adjacent to the BBM [Shiau and Levine, 1980; Shiau, 1990]. This low pH environment increases the critical micellar concentration of fatty acids and cholesterol. The fatty acids become protonated, thereby increasing their hydrophobic and lipophilic properties [Small et al., 1984].

Other important aspects influencing the passive uptake of lipids is the membrane fluidity, more specifically the outer third of the BBM bilayer [Meddings, 1988]. The fluidity of the membranes is influenced by the presence of dietary lipids. For instance, jejunal BBM is exposed to the majority of dietary lipids and presents higher fluidity than the ileal BBM, which is exposed to far less dietary lipids. Other factors influencing the absorption of lipids may involve the translocation of lipids from the outer bilayer to the inner bilayer, which depends on the composition and properties of the two membranes and composition of the lipid to be absorbed [Devaux, 1991; Meddings, 1988; Meddings and Thiessen, 1989].

1.2.b) Protein-mediated lipid uptake

Lipid uptake may also be protein-mediated, as has been evidenced from the following findings: there is a curvilinear relationship between uptake and very low concentrations of fatty acid [Chow and Hollander, 1979; Stremmel, 1988; Schoeller et al., 1995a; Schoeller et al., 1995b]; plasma membrane fatty acid binding proteins have been identified in the BBM of rat intestine such as $FABP_{pm}$, FAT, caveolin-1 and fatty acid transporter protein (FATP) [Stremmel et al., 1985; Londraville, 1996; Abunrad et al., 1993; Schaffer et al., 1994]; the fatty acid uptake is Na^+- and pH-dependent [Stremmel, 1988; Ling et al., 1989 and Schoeller et al., 1995); the fatty acid uptake may be inhibited by using a polyclonal antibody to the $FABP_{pm}$ or a monoclonal antibody to the $FABP_{pm}$ [Stremmel, 1988; Schoeller et al., 1995]; and the use of trypsin, chymotrypsin, pronase or heat treatment inhibit fatty acid uptake [Potter et al., 1989, Schoeller et al., 1995a; Stremmel, 1988].

The dependence of fatty acid uptake on Na^+- and pH reflects the role of the Na^+/ H^+ exchanger (NHE) in the BBM [Schoeller et al., 1995a; Schoeller et al., 1995b]. Experiments performed with oleic acid confirm this dependence. A time-course of oleic acid uptake into rabbit jejunal bush border vesicles was increased markedly in the presence of opposing Na^+ and H^+ gradients [Schoeller et al., 1995a; Schoeller et al., 1995b]. Furthermore, antibody to $FABP_{pm}$ inhibits oleic acid uptake in rabbit jejunal BBM vesicles only in the absence of opposing Na^+ and H^+gradients, This suggests that oleic acid binds passively to the BBM lipids in a protonated form under acidic conditions maintained by the Na^+/H^+-antiporter, and that the $FABP_{pm}$ is a important component of fatty acid uptake under conditions of low luminal pH or absence of Na^+ [Schoeller et al., 1995a; Schoeller et al., 1995b; Stremmel, 1988; Lucas and Mathan, 1989].

Three isoforms of NHE have been described according to their Na^+ affinity and kinetics, their sensitivity to amiloride or other inhibitory analogues, and their regulation by second messengers [Gristein et al., 1989]. The NHE-1 isoform is found in almost all tissues including the BLM of the gut and the NHE-2 isoform is found in the BBM of gut, kidney and adrenal gland. Both isoforms have similar affinity for Na^+, an internal modifier site, equal sensitivity to amiloride, and are stimulated by serum and phorbol esters. However, different sensitivity to the amiloride analogue ethylisopropylamiloride has been reported [Glesson, 1992]. A third isoform called NHE-3 is found specifically in epithelial cells, and it has been isolated in human and rabbit intestine and kidney [Tse et al., 1992].

The intestinal BBM $FABP_{pm}$ is a 40 kd membrane protein found in the BBM of the villus and crypt cells of rat jejunum and ileum and it is a plasma membrane isoform of the mitochondrial aspartate aminotransferase (AspAT) [Stremmel et al., 1985; Besnard and Niot; 2000]. It is functionally and immunologically similar to $FABP_{pm}$ found in hepatocytes, cardiac myocytes and adipocytes [Potter et al., 1987; Sorrentino et al., 1988], but it is different from the family of cytosolic fatty acid binding proteins [Veerkamp et al., 1991; Weisiger, 1999]. This membrane protein was postulated to mediate fatty acid uptake through an active sodium-dependent process, and its kinetics have been described as a Vmax of 2.1 nmoles/min, and a Km of 93 nmoles [Stremmel, 1985; Stremmel, 1988]. However, large amounts of $FABP_{pm}$ antibody were necessary to inhibit the uptake of lipids. Therefore, there is still controversial whether FABP_{pm} is important for lipid uptake [Besnard and Niot; 2000].

Another protein involved in lipid uptake, a 88 kd membrane protein named fatty acid transporter (FAT) was cloned and associated with the sequestration of fatty acids in adipocytes [Abumrad et al., 1993]. FAT expression is restricted in the adult rat to the site of long chain fatty acid absorption, i.e. the small intestine, more specifically the BBM, suggesting a complementary role of these proteins in intestinal fatty acid uptake [Poirier et al., 1996]. A polyunsaturated enriched diet has been demonstrated to induce an increase in FAT mRNA [Poirier et al., 1996]. The mechanism of this dietary fat induction remains to be determined.

In the enterocyte cytosol there are other fatty acid binding proteins responsible for transport of the absorbed fatty acids to their sites of metabolism [Ek et al., 1997; Lucke et al., 1996; Murphy, 1998]. The intestinal FABP_{c} or I-FABP_{c} is present exclusively in the intestine, has a molecular weight of 14-15 kd [Kaikaus et al., 1990; Jolly et al., 1997; Corsico et al., 1998], is observed in mature villus tip cells, and is absent in crypt cells [Iseki and Kondo, 1990; Sweetser et al, 1988], binds palmitic, oleic and arachidonic acids, has a higher affinity for saturated than for polyunsaturated fatty acids, and has a stoichiometry of moles of fatty acids bound to moles of binding protein of 1:1 [Cistola et al., 1989; Kaikaus et al., 1990; Peeters et al., 1989]. In a knock out model of I-FABP_{c} it has been shown that this lipid binding protein is not essential for dietary fat absorption. However, important effects on the lipid metabolism were speculated due to the weight loss that was observed in null I-FABP mice. Therefore, body weight loss observed in null I-FABP mice may suggest an effect of this fatty acid binding protein on lipid metabolism

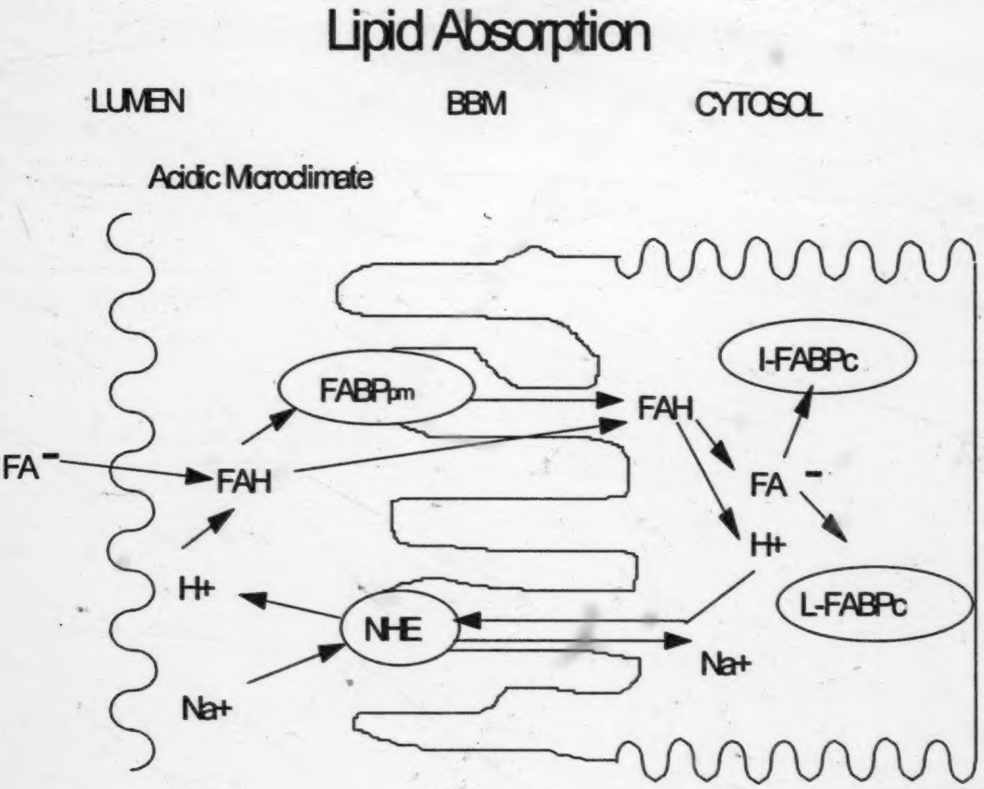
[Holehouse et al., 1998; Vassileva et al., 2000]. The liver $FABP_c$ or L-$FABP_c$ is present in both the liver and the intestine; it has a molecular weight of 14-15 kd [Kaikaus et al., 1990; Jolly et al., 1997], is confined to the crypt-villus junction [Iseki and Kondo, 1990], binds saturated and unsaturated fatty acids as well as monoacylglycerols, lisophospholipids and bile salts, has a stoichiometry of moles of fatty acid bound to moles of binding protein of 1-3:1, and it has a higher affinity for polyunsaturated than for saturated fatty acids [Cistola et al. 1989; Kaikaus et al., 1990; Peeters et al., 1989]. Regulation of expession of L-FABP has been shown to involve fibrate hypoglycemic drugs that strongly bind to peroxisome-proliferator-activated receptors (PPARs). Among the PPAR subtypes, PPARd seems to be the one involved in L-FABP regulation [Niot et al., 1997; Darimont et al., 1998; Poirier et al., 2001]. Other possible modulators of L-FABP and I-FABP expression are leptin, hydrocortisone, insulin and TNF-α [Dube et al., 2001].

The other lipid binding proteins, caveolin-1 is primarily located on BBM and it may be involved in targeted delivery of cholesterol to the endoplasmic reticulum and Golgi apparatus [Conrad et al., 1995]. Finally, FATP4, a member of the FATP family also located on the apical membrane of the enterocyte, has been suggested to be the main fatty acid binding protein involved in the absorption of long chain fatty acids [Stahl et al., 1999]; and acyl-CoA binding protein (ACBP), a cytosolic lipid binding protein has also been implicated in the transport of long-chain acyl-CoA esters, a product of sterification of long-chain fatty acids by acyl-CoA synthetase [Knudsen, 1990; Besnard and Niot, 2000].

Bile acid transport in the terminal ileum is a good example of protein-mediated uptake of lipids [Lewis and Root, 1990; Agellon and Torchia; 2000]. The BBM bile acid transporter is Na^+-dependent and has a higher affinity for unconjugated than for conjugated bile acids. The Na^+-dependent bile acid transporter is a integral membrane glycoprotein that functions in the enterohepatic circulation of bile acids [Gartung et al., 1996; Dawson and Oelkers, 1995; Lester and Ziminiak, 1993; Stieger et al., 1997; Duane et al., 2000]. ILBP, a cytosolic ileal lipid binding protein, acts as an intracellular bile acid carrier, an action that parallels those of I-FABP and L-FABP [Oelkers and Dawson, 1995]. ILBP may work as a regulatory signal for active uptake of bile acids in the ileum, increasing the expression of the Na^+-dependent bile acid transporter when bound to bile acids [Clandinin and Thomson, 2000].

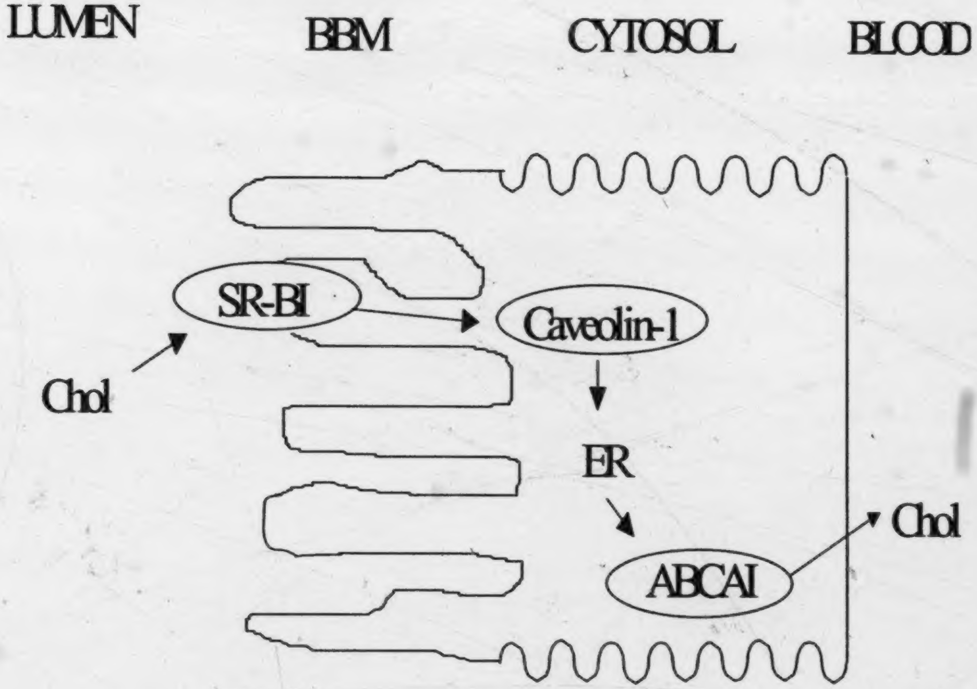
There are also lipid binding proteins responsible for the transport of cholesterol. The scavenger receptor class B type I (SR-BI) is the cholesterol binding protein located on the BBM [Hauser et al., 1998]. This protein facilitates the absorption of dietary cholesterol from both bile salt micelles or phospholipid vesicles. Other lipids such as colestryl esthers, triacylglycerols and phospholipids have also been described to bind to SR-BI [Hauser et al., 1998]. In the cell, cholesterol is transferred to the caveolin-1, an intracellular protein that transports cholesterol from the BBM to the endoplasmic reticulum [Besnard and Niot, 2000]. Finally, another cholesterol binding protein called ATP-binding cassette transporter AI (ABCAI), is responsible for the apolipoprotein A-I mediated efflux of cholesterol from the cell [Oram and Lawn, 2001; Santamarina-Fojo et al., 2001].

Figure 3. Model of Lipid Uptake [Keelan 1998; Schoeller et al., 1995]


Lipid Absorption
LUMEN
BBM
CYTOSOL
Acidic Microclimate
FABPpm
I-FABPc
FAH
FA⁻
FAH
FA⁻
H+
H+
L-FABPc
NHE
Na+
Na+

FA: Fatty acids; FAH: Protonated fatty acids; NHE: Na^+/H^+ exchanger; $FABP_{pm}$: Brush border membrane fatty acid-binding protein; I-$FABP_c$: Intestinal fatty acid-binding protein; L-$FABP_c$: Liver fatty acid-binding protein

Figure 4. Model of Cholesterol Uptake [Hauser et al., 1998; Besnard and Niot, 2000; Oram and Lawn, 2001; Santamarina-Fojo et al., 2001]


LUMEN
BBM
CYTOSOL
BLOOD
SR-BI
Caveolin-1
Chol
ER
ABCAI
Chol

Chol:Cholesterol; SR-BI: Scavenger receptor class B type I; ER: Endoplasmic reticulum; ABCAI: ATP-binding cassette transporter AI

Table 1. Fatty Acid Binding Proteins [Knudsen, 1990; Besnard and Niot, 2000; Stahl et al., 1999; Abumrad et al., 1993]

Fatty Acid Binding Proteins	Molecular Weight	localization	Fatty Acids Transported
BBM $FABP_{pm}$	40 kd	BBM	saturated and polyunsaturated fatty acids
FAT	88 kd	BBM	unknown
caveolin-1	22 kd	intracellular	LCFA and cholesterol
FATP-4	63 kd	BBM	LCFA
I-$FABP_c$	14-15 kd	cytosol; villus tip cells	preferentially saturated fatty acids
L-$FABP_c$	14-15 kd	cytosol; crypt-villus junction cells	preferentially polyunsaturated fatty acids
ILBP	14 kd	cytosol	preferntially bile acids
ACBP	10 kd	cytosol	Long-chain acyl-CoA esters

BBM $FABP_{pm}$: Brush border membrane fatty acid-binding protein
FAT: Fatty acid transporter
FATP: Fatty acid transport protein
LCFA: Long chain fatty acid
I-$FABP_c$: Intestinal fatty acid-binding protein
ILBP: Ileal lipid binding protein
L-$FABP_c$: Liver fatty acid-binding protein
ACBP: acyl-CoA binding protein

2) Intestinal Adaptation

Intestinal adaptation is one of many biological processes where structural and functional modifications occur in response to external or internal stimuli. In other words, intestinal adaptation represents the ability of the intestine to change in response to differences in environmental conditions. For instance, intestinal adaptation improves the nutritional status following the loss of a major portion of the small intestine ("short bowel syndrome"), following chronic ingestion of ethanol, following sublethal doses of abdominal irradiation, in diabetes, with aging, and following fasting and malnutrition [Thomson et al., 1989; Tappenden et al., 1996; Tappenden et al., 1997; Gambara et al., 1997; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Ferraris and Carey, 2000]. Therefore, intestinal adaptation has important implications in survival potential and welfare.

The mechanisms of intestinal adaptation occur at a variety of levels: physiological, cellular and molecular. Most of the signalling processes involved in adaptation are still not well understood. Signals of adaptation may relate to hormone levels, transcription factors, ATP levels or changes in luminal solute concentration [Ferraris and Carey, 2000]. The signals, mechanisms and the adaptive process may be different in the jejunum and ileum, as well as in the crypt and villus tip, explaining the found site-specific alterations and differences between crypt and tip [Thomson and Wild, 1997a; Thomson and Wild, 1997b].

The adaptive process has been defined in terms of transport kinetics, with changes in the value of the maximal transport rate (Vmax) and in the Michaelis affinity constant of nutrients transported actively or by carrier proteins (sugars and amino acids), as well as alterations in the passive permeability coefficient of nutrients transported passively such as short-, medium- and long fatty acids, and cholesterol [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b].

Dynamic morphologic parameters may also adapt. For instance, the crypt cell production rates or the enterocyte migration rates are known to change in some situations of intestinal adaptation [Thomson et al., 1994]. Although both kinetics and dynamic morphologic parameters are altered in the adaptive process, it is much easier to explain the influence of this process on nutrient uptake by kinetic alterations. It is important though that morphological changes be considered when estimating the kinetic parameters of absorption. For instance, morphological changes such as blunting of the mucosal growth and mucosal hyperplasia after intestinal resection are observed when dexamethasone is given subcutaneously [Park et al., 1994].

Many models of intestinal adaptation have been described. For instance, glucose uptake has been found to be increased during pregnancy [Musacchia and Hartner, 1970], lactation [Cripps and Willians, 1975], high carbohydrate intake [Diamond and Karasov, 1984; Sanderson and Naik, 2000], hyperglycemia [Csaky and Fisher, 1981; Fischer and Lauterbach, 1984; Maenz and Cheeseman, 1986], after intestinal resection [Glesson et al., 1972; Robinson et al., 1982], and diabetes [Keelan et al., 1985; Keelan et al., 1987]. Aging has been associated to an increase in the protein-mediated transport of hexoses and a decrease in the passive uptake of lipids [Bowman and Rosemberg, 1983; Hollander and Morgan, 1979; Tolozoa and Diamond, 1992; Thomson, 1979, 1980, 1981]. Diabetes mellitus increases both protein-mediated and passive transport of sugars [Fedorak et al., 1987; Thomson and Rajotte, 1983]. External abdominal irradiation and chronic ethanol consumption decrease both active and passive components of uptake [Thomson et al., 1983, 1984]. Most transporters are up-regulated by dietary substrate levels, however toxic substances and two essential amino acids do not exert the same effect [Diamond and Karasov, 1987]. These examples illustrate the diversity and variability of this adaptive process.

Phospholipid, cholesterol and fatty acid composition of plasma membranes may be modified in mammalian cells [Spector and Yorek, 1985; Korotkova and Strandvik, 2000]. These changes in the membrane lipid composition may affect the physical properties of the membrane, which can alter and influence the activity of membrane-bound proteins as SGLT1, GLUT2, GLUT5 and other transporters. For example, changes in the BBM fluidity have been demonstrated to influence the passive uptake of lipids as well as the carrier-mediated D-glucose uptake [Brasitus et al., 1989; Meddings, 1988, 1990; Meddings and Thiessen, 1989; Hyson et al., 1997]. Enhancement of fluidity increases the uptake of lipids. However, fluidization of BBM from enterocytes located on the villous tip decreases the uptake of D-glucose to levels seen in the BBM from enterocytes located on the crypts [Meddings, 1990].

Dietary constituents provide a continuous signal for intestinal adaptation [Thomson et al., 1986; Jump and Clarke, 1999; Sanderson and Naik, 2000]. Every day enterocytes are exposed to different nutrients which vary according to their intake and availability. For that reason, the intestine must be able to adapt as soon as possible to variations in the dietary load and composition [Diamond, 1991; Sanderson and Naik, 2000]. The exposure to different diets appear to have importance when occurred at young age, and this concept has led to the theory of "critical period programming" [Karasov et al., 1985; Keelan et al., 1990; Perin et al., 1999]. This periods is defined as "a biological mechanism which is turned irreversibly on or off only once during an individual's lifetime in response to conditions prevailing at some critical stage" [Karasov et al., 1985].

Changes in villus height, BBM lipid composition, nutrient transport and BBM enzyme activity in response to alterations in the dietary macronutrient content (carbohydrate, cholesterol, essential fatty acids and protein) have been demonstrated [Thomson and Rajotte, 1983; Keelan et al., 1987, 1990; Waheed et al., 1998; Perin et al., 1999]. For example, animals fed PUFA enriched diets have a decline in glucose uptake as compared to animals fed SFA enriched diet [Thomson et al., 1987; Thiesen et al., 2000]. Animals fed a glucose-enriched diet have increased glucose uptake, resulting from up-regulation of both BBM and BLM glucose transporters [Cheeseman and Maenz, 1989; Cheeseman and Harley, 1991, Ferraris et al., 1992]. A fructose- enriched diet also results in enhancement of fructose uptake and increased expression of GLUT5 without changing glucose uptake [Burant and Saxena, 1994; Shu et al., 1997; Monteiro and Ferraris, 1997]. Both substrates glucose and fructose are specific in terms of up-regulation of their correspondent transporters, SGLT1 and GLUT5. Therefore, changing the composition of the diet, modifications in the transport of nutrients are expected.

2.1) Patterns and Mechanisms of Adaptation

The alterations in the cell kinetics that result in modification of the nutrition status may be specific or non-specific. Non-specific mechanisms involve kinetic alterations that result in changes in the intestinal mucosal mass and or villous surface area, leading to changes in the uptake of all nutrients, including those absorbed passively [Rand et al., 1993]. On the other hand, specific mechanisms involve up- or down-regulation of specific transporters responsible for the uptake of particular nutrients [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b].

2.2) Dietary Lipids

Early exposure of weaning rabbits to a high-cholesterol diet for 6 weeks leads to an increase of acyl-CoA-cholesterol acyltransferase (ACAT) activity [Subbish et al., 1989]. The persistence of this enhanced capacity to form cholesterol esters is evidenced when these animals are re-exposed to high-cholesterol diets after being fed a normal diet for nine weeks. Therefore, depending upon early dietary lipid experience, there may be a different amount of cholesterol absorbed in later life. This fact brings up the possibility that lipid absorption and metabolism may be influenced genetically by a process that is modulated by early childhood events involving nutritional experiences.

Currently the typical North American diet contains up to 34 % of fat [NHANES III, 1993; Taubes, 2001]. Several kinds of cancer and cardiovascular diseases are associated with the intake of high dietary fat. More specifically, cholesterol and saturated fats are highly involved in the process of pathogenesis of many disorders as well as cardiovascular diseases. On the other hand, polyunsaturated fats are thought to be beneficial in decreasing the incidence of these pathological processes. Further benefits such as a lower elevated serum triglyceride and cholestrol levels may be observed with a diet enriched in ω3 polyunsaturated fatty acids as compared with a diet rich in ω6 polyunsaturated fatty acids [Bang et al., 1980; Carrol, 1986, Schectman et al., 1989].

BBM total lipid composition is not changed significantly in animals fed diets of different fatty acid composition with the changes [Keelan et al., 1990; Thomson et al., 1986, Thomson et al., 1987]. However, the BBM phospholipid fatty acid composition is modulated by dietary lipids [Keelan et al., 1990; Calmp et al., 1997]. In other models of intestinal adaptation such as streptozocin-induced diabetes, chronic ethanol feeding, intestinal resection, starvation, aging and following external abdominal irradiation, the BBM total phospholipid content composition changed, and may contribute to the adaptive alterations in these models [Keelan et al., 1985, Keelan et al., 1990; Waheed et al., 1998]. Therefore, the adaptive alterations in nutrient transport observed with dietary lipids are not fully explained by alterations in the BBM lipid composition.

Feeding diabetic rats a high fat diet enriched in ω6 polyunsaturated fatty acids results in a decrease in the intestinal sugar uptake and an improvement in the clinical control of the diabetes [Rajotte et al., 1988; Thomson et al., 1987; Thomson et al., 1988]. A further negative effect on intestinal sugar uptake is again observed with diets enriched in ω3 polyunsaturated fatty acids [Thomson et al., 1988]. Therefore, a diet enriched in polyunsaturated fatty acids may be the important in the care of diabetic patients.

The effects of dietary lipids on another model of intestinal adaptation, 50% small intestinal resection, have also been published [Keelan et al., 1996]. Once again negative effects on intestinal uptake were obtained with those animals fed the polyunsaturated diet, while those fed the saturated diet had a jejunal uptake of D-glucose twice as high in those fed the polyunsaturated diet. In this case, it would be beneficial to prescribe a diet rich in saturated fatty acids for those patients who have undergone an intestinal resection [Allard and Jeejeebhoy, 1989; Woolf et al., 1983].

The mechanisms by which dietary lipids may mediate changes in membrane structure and function are not well understood. Dietary fatty acids are modulators of BBM phospholipid fatty acid composition [Keelan et al., 1990; Clamp et al., 1997]. A diet enriched with saturated fatty acids is associated with increases in the saturation of BBM phospholipid fatty acids, while a diet enriched with ω6 polyunsaturated fatty acids is associated with an increase in the unsaturated fatty acids in the BBM phospholipids [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b]. The degree of fatty acid unsaturation or saturation, as well as the cholesterol content, are factors that influence the fluidity of the BBM [Alberts et al., 1994]. Changes in the fluidity of the membrane may alter the permeation of molecules and nutrients through this barrier, as well as the conformation of binding sites on proteins as SGLT1, GLUT5 [Meddings, 1990].

The dietary changes in the membrane lipid composition may be modulated by alterations in enterocyte microsomal membrane desaturase activity [Keelan et al, 1994]. By changing the enzymes involved in lipid synthesis, dietary lipids would subsequently alter the lipid composition of the membrane. Interestingly, BBM cholesterol does not change in response to dietary lipids, suggesting that the BBM cholesterol is tightly controlled [Keelan et al., 1994]. However, the enterocyte microsomal membrane cholesterol level is influenced by diet and endogenous synthesis [Keelan, 1998].

Alterations in enterocyte microsomal membrane lipid composition do not always explain the alterations found in the BBM lipid composition [Keelan et al, 1985]. There may be heterogeneity of the lipids along the villus, and studies done with mixtures of cells collected from the entire villus may obscure alterations occurring at just one portion of the villus. Alternatively, there may be some modification of the phospholipid fatty acids after the lipids leave the microsomes of the enterocyte and traffic to the BBM.

The way by which dietary lipids alter gene expression and consequently change membrane compositon and/or nutrient transport is not clear, but it has been proposed to be through activation of peroxisome proliferator-activated receptors (PPAR), hepatic nuclear factor-4 (HNF-4), nuclear factor k B (NFkB) and sterol response element binding proteins 1c (SREBP1c) [Jump and Clarke, 1999]. Binding to these transcription factors, dietary lipids would affect the rate of transcription and consequently protein synthesis of nutrient transporters [Jump and Clarke, 1999; Poirier et al., 2001].

2.3) Enterocyte Microsomal Membranes

Once in the cytosol, the absorbed free fatty acids are transported to the endoplasmic reticulum by the fatty acid binding proteins (see section 1.2.b), where they are used for re-synthesis of triacylglycerols, re-synthesis of phospholipids, and esterification of cholesterol. These re-synthesized and newly synthesized lipids are then directed to membrane synthesis or lipoprotein assembly [Keelan et al., 1994].

The first step of fatty acid metabolism involves the acyl-CoA synthetase action that converts these free fatty acids into acyl-CoA. This acyl-CoA may now be used for reesterification of absorbed cholesterol and lysophosphatidylcholine, for synthesis of phosphatidic acid, and for synthesis of diacylglycerol. The diacylglycerol may be used for the synthesis of triacylglycerols and/or phospholipids by the action of choline and ethanolamine phosphotransferases [Brindley and Hubscher, 1966; Ohkubo et al., 1991].

In relation to phospholipid synthesis, two enzymes are important: one is the CTP:phosphocholine cytidyltransferase enzyme that converts phosphocholine to CDP-choline. This conversion that is the rate-limiting step in the synthesis of phosphocholine, the most abundant phospholipid found in many membranes, including intestinal BBM [Pelech et al., 1983a; Pelech et al., 1983b]. The other enzyme is the phosphatidylethanolamine N-methyltransferase that converts phosphatidyethanolamine to phosphocholine [Bremer and Greenberg, 1961].

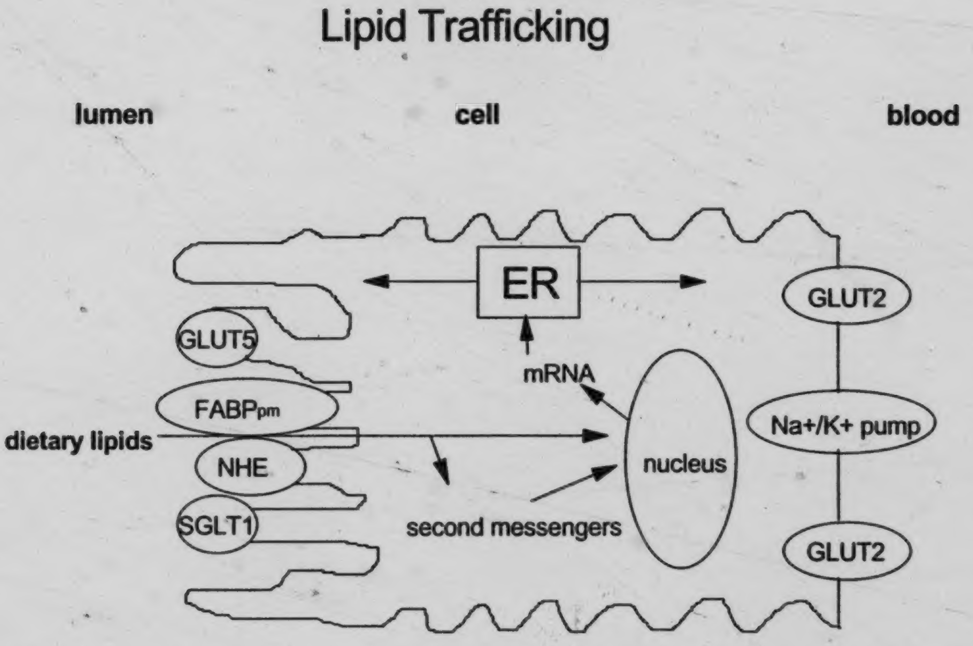
Free fatty acids are also desaturated and elongated in the endoplasmic reticulum to form long-chain polyunsaturated fatty acids [Strittmatter and Rodgers, 1975; Holloway and Holloway, 1975, 1977]. The conversion of linoleic acid (18:2ω6) to γ-linolenic acid (18:3ω6), and α-linoleic acid (18:3ω3) to 18:4ω3, is performed by Δ6-desaturase. This is the rate-limiting step in the synthesis of arachidonic acid (20:4ω6) and eicosapentanoic acid (20:5ω3) [Sprecher, 1981]. Desaturases are endoplasmic reticulum intrinsic membrane proteins that remove two hydrogen atoms from acyl-CoA derivated from exogenous or endogenous sources, forming a cis double bond [Jeffcoat, 1979; Rodgers and Strimatter; 1973, 1974]. Desaturase activity may influence or regulate the differences found in the fatty acid composition found in jejunal and ileal enterocyte microsomal membranes (EMM) and BBM [Garg et al., 1990; Keelan et al., 1990].

Dietary lipids may produce changes in the lipids in EMM and BBM, as well as BBM uptake. This results from unknown signals that affect the activities of EMM lipid enzymes, or interfere in the gene expression of EMM lipid enzymes and BBM transporters. Changes in enterocyte microsomal enzyme (EMM) activities may be mediated by dietary lipids acting to modify signal transduction by second messengers such as cAMP, Ca^{+2} and diacylglycerol, altering RNA expression [Keelan et al., 1994]. This process involves peroxisome proliferator-activated receptors (PPAR), hepatic nuclear factor-4 (HNF-4), nuclear factor k B (NFkB) and sterol response element binding proteins 1c (SREBP1c) [Jump and Clarke, 1999].

The lipids present in the diet may also influence lipid synthesis. During phospholipid synthesis, ω3 fatty acids compete with ω6 fatty acids for the sn-2 position of phospholipids, resulting in the enrichment of ω3 fatty acids in phospholipids [Keelan, 1998]. Long chain fatty acids may also serve as substrates for desaturases and may be desaturated and elongated to form long-chain polyunsaturated fatty acids [Keelan, 1998].

If dietary lipids do affect gene expression and do it through activation of PPARs, there is a strong possibility of interaction of lipids with other ligands of this receptor. It is also known that PPARs belong to the superfamily of receptors that include the glucocorticoid receptor [Huin et al., 2000]. This provides support for the possibility of dietary lipids interacting with the glucocorticosteroid receptor and affecting the expected steroid effect. This speculation provides the basis for analysis of the interaction between glucocorticosteroids and dietary lipids.

Figure 5. Lipid Trafficking [Keelan et al., 1998; Jump and Clarke, 1999; Poirier et al., 2001]


Lipid Trafficking
lumen
cell
blood
ER
GLUT2
GLUT5
mRNA
FABPpm
Na+/K+ pump
dietary lipids
NHE
nucleus
SGLT1
second messengers
GLUT2

Possible second messengers:

- peroxisome proliferator-activated receptors (PPAR),
- hepatic nuclear factor-4 (HNF-4),
- nuclear factor k B (NFkB)
- sterol response element binding proteins 1c (SREBP1c)

2.4) Short Bowel Syndrome

The topic of short bowel syndrome (SBS) in children and adults has been reviewed [Sturm et al., 1997; Vanderhoof et al., 1997]. There is a quantitative relationship between the load and the capacity in adaptively regulated physiological systems. Humans can tolerate an approximately 50% resection of the small intestine without developing clinically impaired digestive function, but more extensive resection leads to the SBS. The most frequently used animal model for studying SBS, the rat, can tolerate approximately 80% resection. When the compensatory regrowth of the small intestine is exceeded, such as occurs with a 70% resection in mice, the animal does not survive [Hammond et al., 1996]. Markers of this adaptive response have been suggested to be the expression of SGLT1 and plasma citrulline concentration, but further work has not confirmed these conclusions [Sigalet and Martin, 1998; Crenn et al., 2000]. Assessment of glucose transport using 3-O methylglucose (4-hour urinary recovery of sugars using HPLC) may be useful in assessing the absorptive capacity of the remaining gut after intestinal resection [Debru et al., 2001].

The small bowel adaptation that occurs after intestinal resection is due to luminal nutrients, hormones and pancreatobiliary secretions [Thompson et al., 1999; Ghatei et al., 2001]. The trophic effect on the intestinal mucosa of peptides such as growth hormones (GH), neurotensin (NT), gastrin-releasing peptide (also known as bombesin) and epidermal growth factor (EGF) has been demonstrated [Erwin et al., 1999]. In the suckling rat, NT enhances the intestinal proliferative phenomenon, but does not improve the course of medium term postresection growth [Lopez et al., 1997]. NT also increases the adaptive intestinal process after colon resection and reduces plasma enteroglucagon-like immunoreactivity in rat [Mata et al., 1997].

In a randomized, six-week, double blind, placebo controlled cross-over study, eight patients with SBS were administered GH, oral glutamine and a high carbohydrate/low fat diet. Active treatment transiently increased body weight, increased the absorption of sodium and potassium, and decreased gastric emptying; unfortunately, the assimilation of macronutrients, stool volumes and morphometry of the small bowel mucosa were unchanged compared with those of patients who received placebo [Scolapio et al., 1997]. GH administration has also been demonstrated to increase acutely sugar absorption [Tavakkolizadeh et al., 2001]. Further studies using a 85% mid-small bowel resection in Sprague-Dawley rats did not confirm the beneficial effects of glutamine supplementation [Zhou et al., 2001]. However, glutamine administration enhanced the gut-trophic effect of GH [Zhou et al., 2001]. Furthermore, a systematic review of the all the evidence published so far in the literature concludes that the benefit of GH alone, or in combination with glutamine is, if any marginal [Li-Ling and Irving, 2001; Jeppensen et al., 2001; Ljungmann et al., 2000; Skudlarek et al., 2000]. Finally, a double blind study in eight short bowel syndrome patients for 5 days did not show any improvement of growth hormone and parenteral glutamine in intestinal absorption [Szkudlarek et al., 2000]; and supplementation of glutamine for 8 weeks did not show any improvement in gut function [Scolapio, 2001]. When analysing the studies involving GH and glutamine supplementation, one should consider the model used, the parameters used to measure response and the timing. Most of the positive studies measure a acute response of GH that in fact it seems to be present, but when more time is given, no evidence of the beneficial supplementation of GH and/or glutamine is observed.

In a rat model of SBS (70% jejunal-ileal resection), no evidence was found that the combination of glutamine and GH enhanced mucosal mass, protein, DNA levels or sucrase activity [Vanderhoof et al., 1997]. GH has a non-specific effect on small intestinal growth. GH is released by arginine; in a rat model of small bowel resection, the administration of L-arginine had no effect on food intake, body weight or plasma insulin-like growth factor (IGF)-1 levels [Hebiguchi et al., 1997]. The administration of EGF or IGF-1 after massive small bowel resection in rats does not modify villous length, crypt length or the villous-to-crypt ratio [Lukish et al., 1997]. However, significant increases in weight gain and glucose transport have been demonstrated in rats undergoing a massive intestinal resection and treated with GH, IGF-1 and GLP-2 [Sigalet and Martin, 2000; Thulesen et al., 2001; Ljungmann et al., 2001]. It is important to note that when analysing differences in the diverse studies looking at intestinal resection, one should consider the site, extension and doses of the agents administered.

Provision of a liquid diet, using a small number of sutures for the anastomosis, and resection of no more than 50% of the proximal small intestine are important for the survival of the mouse following small bowel resection [Helmrath et al., 1996]. Segmental reversal of the small bowel may reduce parenteral nutrition dependency in patients with SBS [Panis et al., 1997]. Therefore, surgical aspects are also important when analysing the adaptive response after intestinal resection.

During early adaptation (after three days) in the remnant small intestine in rats, the genes involved in nutrient trafficking, protein processing and cell cycle regulation are transcriptionally regulated in the residual small intestine. No observations have been made when looking 2 weeks or 4 weeks after intestinal resection. This regulation occurs in distinct temporal and regional patterns consistent with a complex multifaceted response to intestinal resection [Dodson et al., 1996]. Following small bowel resection, a number of genes identified by cDNA microarray analysis are induced in the adapting remnant [Erwin et al., 2000; Stern et al., 2001]. Epimorphin/syntaxin 2 mRNA that codes for a membrane-associated protein involved in morphogenesis of the lungs nad skin; PC4/TIS7, a gene involved in nerve growth factor-mediated cytodifferentiation; rsTPI, a putative protein of 254 amino acids highly homologous to triosephosphate isomerase; and a secreted protein, acidic and rich in cysteine (SPARC), a counteradhesive matricellular glycoprotein that may be involved in signal transduction and communication among enterocytes; ileal remnant repressed (IRR)-219, a mucinlike intestinal protein exclusively produced by goblet cells; and bcl-2 gene product, an antiapoptotic gene may be among other signals that may be involved in the adaptive response after intestinal resection [Goyal et al., 1998; Rubin et al., 1998; Wang et a;., 1999; Puolakkainen et al., 1999; Rubin et al., 2000; Welters et al., 2000]. Special consideration should be given when considering timing of expression of these particular signals.

Luminal nutrients (such as glutamine, soluble fibres and short-chain fatty acids), intestinal hormones (such as GH, EGF, enteroglucagon and IGF-1), and pancreatobiliary secretions are important for the process of intestinal adaptation [Falcone et al., 2000; Benhamou et al., 1997; Prasad et al., 2000; Avissar et al., 2000; Topstad et al., 2001;

Durant et al., 2001; Welters et al., 1999; Secor et al., 2000; Fitzgerald et al., 2001; Ljungmann et al., 2001].

Polyamines are required for the normal repair of intestinal damage. The inhibition of ornithine decarboxylase (ODC), the rate-limiting enzyme of polyamine biosynthesis, adversely affects the normal healing process [Segovia-Silvestre et al., 2001]. Polyamines are important for cell attachment and expression of the integrin alpha$_2$beta$_1$, a putative receptor for collagen and laminin [Santos et al., 1997]. The impairment of protein cross-linking, and the inhibition of the expression of cell surface receptors that bind extracellular matrix proteins impairing communication and transduction among enterocytes, may be part of the mechanism by which polyamine deficiency retards cell migration in the small intestine. Absence of matrix proteins or the signalling activated by these proteins result in physiological cell death called anoikis, illustrating the role of matrix proteins on integrity of the tissue architecture [Rosen et al., 2000]. Ornithine α-ketoglutarate a precursor of polyamines enhances intestinal adaptation after intestinal resection, improving muscle glutamine and protein content [Dumas et al., 1998], however, the administration of *Saccharomyces boulardi*, a yeast that synthesizes polyamines, does not improve the adaptive response after a 80% jejunoileal resection in rats [Kollman et al., 2001]. Increases in plasma cortisol level (administration of hydrocortisone for two days) have been shown to stimulate polyamine synthesis and consequently ODC activity [Wu et al., 2000]. However, no data is available regarding the chronic use of steroids and the use of locally acting glucocorticosteroids.

The amino acid glutamine stimulates mucosal growth and promotes intestinal adaptation although some controversy as mentioned before is evident. When glutamine is coupled with growth factors such as GH, IGF-1, glucagon-like peptide-2 (GLP-2) or IL-11, a high level of bowel adaptation can be obtained [Willmore, 1997; Gu et al., 2001]. Glutamine stimulates the induction of ODC, and glutamine activates extracellular signal-regulated kinases (ERKc) and Jun nuclear kinases, resulting in increased activating protein 1-dependent gene transcription (glutamine ➔ ODC ➔ ERK ➔ AP-1) [Rhoads et al., 1997]. After intestinal resection, IL-11 by itself improves absorptive capacity for galactose and glycine beyond the normal adaptive response increasing the hypertrophic and hyperplasic responses [Alavi et al., 2000, Dickinson et al., 2000]. The mechanism involved in this adpative response is unknown.

The topic of molecular aspects of mucosal repair has been reviewed [Moss and Wright, 1996], as has the topic of epithelial cell growth and differentiation [Robine et al., 1997]. The superfamily of G protein-coupled receptors includes receptors for peptide and nonpeptide hormones and neurotransmitters. A common functional feature of cellular responses to G-protein coupled receptor agonists is that they are rapidly attenuated. This downregulation is characterized by the depletion of the cellular receptor content due to alterations in the rate of receptor degradation and synthesis [Grady et al., 1997].

It is unknown why different genes are expressed in the proximal versus the distal intestine. This may arise from specific variations in the transcription factors that interact with the promotor and the enhancer regions of these genes. Homeodomain transcription factors (known as homeobox genes) are involved in establishing gradients of differentiation during development, as well as maintaining these patterns of expression through continued expression in adult tissues. The homeobox gene product Cdx-2 interacts with the promoters for several genes expressed in the intestine, such as BBM sucrase-isomaltase. Many homeobox genes are expressed in adult human intestinal mucosa, and some are found predominantly in one region [Walters et al., 1997].

Protein kinase C (PKC) is a family of serine/threonine kinases that play a central role in signal transduction . PKC has been implicated in the control of cell growth, differentiation and transformation. PKC activation initiates a signalling cascade, leading to alterations in gene expression and modulation of a variety of cellular functions. PKC also may be involved in the inhibition of cell growth and differentiation. PKCα is implicated in the negative regulation of intestinal epithelial cell growth both in *vitro* and in *situ* via pathways that involve modulation of Cip/Kip family cyclin-dependent kinase inhibitors [Frey et al., 1997]. PKC II β could be involved in the signalling involving SGLT1 and GLUT2 mobilization from the BLM to the BBM [Kellet, 2001].

Members of the src family of the nonreceptor protein tyrosine kinases play a role in the proliferation and differentiation of cells. The catalytic domain of the src-related protein tyrosine kinase has been cloned. Complete nucleotide sequencing of the gastrointestinal-associated tyrosine kinase has been demonstrated [Sunitha et al., 1996]. In the rat intestine, gastrointestinal-associated tyrosine kinase plays a specialized role in transduction of signals and activation of pathways involved in the growth and differentiation of gut columnar epithelial cells [Sunitha et al., 1996].

The immediate-early response genes are a group of genes that rapidly increase after various mitogenic and other pathologic stimuli. The protein products of some of these genes act at the transcriptional level to initiate downstream events that are believed to culminate in cellular proliferation. Nup 475 and c-jun are two intermediate-early genes that are found in the nucleus. mRNA levels of both Nup 475 and c-jun are markedly increased 2 hours and 24 hours following a 70% proximal small bowel resection in rats [Ehrenfried et al., 1995]. Other immediate-early response gene such as c-myc and c-fos have also been demonstrated to be involved in the processes of intestinal adaptation [Tappenden et al., 1998].

Human intestinal muscle produces produce IGF-1, and both TGF-α and TGF-β1 in a time-dependent reciprocal fashion that parallels their effect on growth [Kuemmerle, 1997]. GH induces IGF-1 synthesis, and IGF-1 may play an important role in the regulation of intestinal growth and maturation [Gillingham et al., 2000]. IGF-1, or GH plus IGF-1, increase intestinal growth parameters in rats fed by total parenteral nutrition (TPN), whereas GH alone has no effect [Peterson et al., 1997; Peterson et al., 2000]. IGF-1 has been shown to improve the intestinal absorption of nutrients by enhancing the activity of the Na^+/K^+ ATPase, that provides the gradient utilized for the absorption of glucose and amino acids, by a mechanism that may involve phosphatidylinositol 3-kinase [Alexander and Carey, 2001].

There is a bidirectional network among the neural, endocrine and immune systems. IL-1β, IL-6 and TNF-α are cyotkines polypeptides that are predominantly produced by monocytes and macrophages. They also stimulate the corticotropin-releasing factor containing cell, and thereby activate the pituitary-adrenal axis. These cytokines are present in the intestinal mucosa and may be modulators of intestinal function. The polyamine spermine when given by mouth to neonatal rats increases the plasma concentration of IL-1β, IL-6 and TNF-α [Kaouass et al., 1997]. Spermine is the most effective polyamine in regulating ODC activity [Yuan et al., 2001]. This polyamine induces post-natal intestinal development and corticosterone secretion through a

7

cytokine-dependent mechanism. The administration of spermine to neonatal mice increases the intestinal length, decreases lactase activity and increases sucrase activity, mimicking the normal ontogenic development of the intestine. These findings with spermine are the same when glucocorticosteroids are administered. Spermine also increases the percentage of intra epithelial lymphocytes (IEL) expressing TCRαβ, CD4, CD5 and CD54, which ones represent the phenotypes of more mature lymphocytes. This indicates that oral spermine given to neonatal mice also results in precocious maturation of the murine intestinal immune system [ter Steege et al., 1997]. This suggests that there is a role for polyamines, cytokines and glucocorticosteroids in the adaptive intestinal response that occurs after intestinal resection.

The cytokine signalling from the membrane to the nucleous in the intestine has been shown to involve the Janus kinase (JAK)-signal transducers and activators of transcription (STAT), nuclear factor κ β, mitogen activated protein kinases (MAPK), sphingomyelinase-ceramide, and phosphattidylinositol 3'-kinase [Derek et al., 2000]. Therefore, all these molecular signals may be involved in the adaptive response obtained with cytokines.

Leptin, a peptide produced by adipocytes and physiologically involved in regulation of satiety and body fat, has been identified to work as a growth factor in a 80 % small bowel resection model in Sprague Dawley rats. One week after resection these rats were infused leptin through a jugular venous catheter for 14 days. Leptin improved the gut response measured by galactose absorption and GLUT5 expression after intestinal resection, suggesting possible involvement of this hormone in the adaptive response after intestinal resection. [Pearson et al., 2001].

3) Glucocorticosteroids

3.1) Introduction

Thus far in the literature review, I have considered normal mechanisms of sugar and lipid abosprtion, and I have reviewed the topic of intestinal adaptation, including the topic of intestinal resection or short bowel syndrome. Because glucocorticosteroids have been implicated as possibly being important in the ontogeny of intestinal development, as well as possibly implicated in intestinal adaptation; and because glucocorticosteroids affect the intestine and there is a strong possibilty of interaction with dietary lipids, I developed hypotheses related to intestinal adaptation, glucocorticosteroids, dietary lipids, intestinal resection, and intestinal nutrient transport. In this section of my literature review I provide a summary of the effects of glucocorticosteroids on the intestine [Batt and Peters, 1976a; Batt and Peters, 1975; Scott et al., 1980; Scott et al. 1981; Batt and Scott, 1982; Murphy, 1982; Park et al., 1994; Spitz et al., 1994; Guo et al., 1995; Marti and Fernandez-Otero, 1994; McDonald and Henning, 1992; Lebenthal et al., 1972; Nsi-Emvo et al., 1996; Sangild et al., 1995]. For sake of completeness, I will also consider the effect of glucocorticosteroids on other body systems.

There is a large number of bioactive hormones secreted from the adrenal cortex. The hormones secreted in significant amount are: hydrocortisone or cortisol, corticosterone and aldosterone. All three have mineralocorticoid activity, and cortisol and corticosterone have glucocorticoid activity [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland J, 1980; Bowman and Rand, 1980]. Glucocorticosteroids are a type of corticosteroids that have potency based on liver glycogen deposition. The major naturally occurring glucocorticosteroid in man is cortisol [Baxter, 1979; Peterson and

Pierce, 1960], and in rats and mice the major glucocorticosteroid is corticosterone [Shimizu et al., 1983]. Cortisol accounts for approximately 95 % of all glucocorticoid activity in humans [Haynes and Murad, 1985, Haynes and Larner, 1975; Crossland, 1980; Baxter, 1979].

3.2) Historic Perspectives

Cortisone was the first corticosteroid to be discovered in 1935 by Edward C. Kendal of the Mayo Foundation for Medical Education and Research. Cortisone was initially called compound E [Aviado, 1972]. In 1946, Sarret partially synthesized cortisone [Aviado, 1972; Baxter, 1979]. Adrenal extracts containing cortisol had been used in therapy even earlier [Baxter, 1979; Ehrich et al., 1992]. In 1948, the 37 steps in the synthesis of cortisone were elucidated [Aviado, 1972; Laurence and Bennet, 1984]. In the same year, compound E was named cortisone [Aviado, 1972].

In 1946 Hennch undertook the first therapeutic use of glucocorticosteroids in persons with rheumatoid arthritis, with dramatic results [Crossland, 1980; Aviado, 1972; Laurence and Bennet, 1984]. Shortly afterwards corticosteroids were introduced to treat asthma. Since 1950, the glucocorticosteroids have been one of the mainstays in the therapy of active ulcerative colitis and Crohn's disease [Brattsand, 1990].

3.3) Biosynthesis

Corticosteroids are synthesyzed from cholesterol. Part of this cholesterol is from the diet [Haynes and Murad, 1985; Haynes and Larner, 1975; Bowman and Rand, 1980]. Another portion of cholesterol is synthesized and stored within the adrenal glands [Bowman and Rand, 1980]. This biosynthesis is called corticosteroidogenesis. It occurs at the inner mitochondrial membrane at rest or following the administration of adrenocorticotrophic hormone (ACTH) [Stocco and Clark, 1996; Simpson et al., 1979]. In fact the rate-limiting step of biosynthesis of glucocorticosteroids is the delivery of the substrate, cholesterol, to the inner mitochondrial membrane. Many proteins such as sterol carrier protein 2 (SCP2), steroidogenesis activator protein polypeptide (SAP), peripheral benzodiazepine receptor (PBR), and steroidogenic acute regulatory protein (StAR) have been described to regulate the transport of cholesterol to the inner mitochondrial membrane [Stocco and Clark, 1996].

Release of cortisol or corticosterone from the adrenal cortex is regulated by the hypothalamic-pituitary-adrenal axis, and involves initial secretion of corticotropin-releasing hormone from the hypothalamic corticotropin releasing centre and consequently secretion of ACTH from the pituitary gland that finally leads to secretion of cortisol or corticosterone from the adrenal cortex [Williams and Dluhy, 1998]. When the administration of ACTH does not result in an increase in cortisol (major human glucocorticosteroid) or corticosterone (major rat glucocorticosteroid) blood concentrations, there is inhibition of the hypothalamic-pituitary-adrenocortical axis. The use of synthetic glucocorticosteroids frequently blocks the ACTH response. A stimulatory action of free fatty acids (such as oleic and linoleic acids) on corticosteroidogenesis has been proposed. This modulation of corticosteroidogenesis by these naturally occurring lipids may be an important component of the control mechanisms within the hypothalamic-pituitary-adrenocortical axis in disorders of lipid homeostasis, such as obesity, starvation or diabetes [Sarel and Windmaier, 1995].

Adrenocortical cells have large numbers of receptors that mediate the uptake of low density lipoprotein (LDL), the main source of cholesterol [Haynes and Murad, 1985;

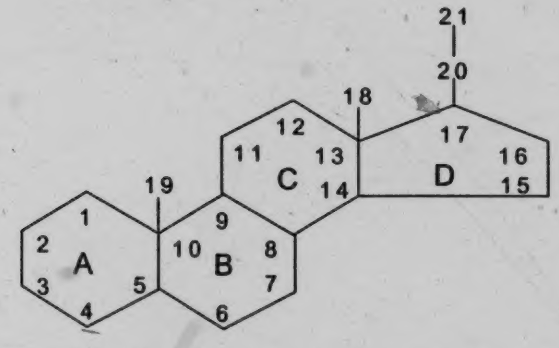
Haynes and Larner, 1975; Myles and Daly, 1974; Simpson and Mason, 1979; Cope, 1972; Rainey et al., 1992]. The reactions catalyzed by mixed-function oxidases that contain cytochrome P-450, and require NADPH and molecular oxygen, convert cholesterol to 21-carbon corticosteroids [Haynes and Murad, 1985; Haynes and Larner, 1975; Simpson and Mason, 1979; Bowman and Rand, 1980; Nonaka Y. et al., 1995]. Many reactions involved in steroid metabolism are hydroxylation reactions, in which one atom of oxygen is inserted into the hydroxyl group of the product, and the other is reduced to water. These reactions are called mixed function oxidase [Simpson and Mason, 1979], and consist of 17α-hydroxylation to form Δ17α-hydroxyprogesterone, 5-3β-hydroxyl configuration to Δ4-3 keto configuration to form progesterone, 21-hydroxylation to form 11-desoxycortisol, and 11-β-hydroxylation to form cortisol [Bowman and Rand, 1980]. The forms of rat CYP11B genes CYP11B1 and CYP11B2 encode steroid 11 beta-hydroxylase and aldosterone synthase, respectively [Mukai et al., 1993]. The products of corticosteroidogenesis are not stored in adrenal tissue, since the rate of biosynthesis is equal to the rate of secretion [Haynes and Murad, 1985; Haynes and Larner, 1975; Simpson and Mason, 1979; Bowman and Rand, 1980].

3.4) Chemical Structure

Steroids are classified as lipids, because of their greater solubility in organic than in aqueous solvents. As the number of hydroxyl or carbonyl groups on the steroid nucleus increases, so does its solubility in water [Myles and Daly, 1974]. For example, budesonide, a steroid where a 17α ester substitution is introduced, is 100-fold more water soluble than other steroids. This facilitates budesonide dissolution and transport into the bowel wall [Brattsand, 1990].

Glucocorticosteroids are adrenocortical steroids with 21 carbon atoms, because all bear two carbon atoms, disposed in four rings: A, B, C and D. The carbon skeleton is named cyclophenantrene nucleus (i.e., has 17 carbon atoms) [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980; Cope, 1972; Myles and Daly, 1974; Phillips, 1976; Dluhy et al., 1975; Applezweig, 1962].

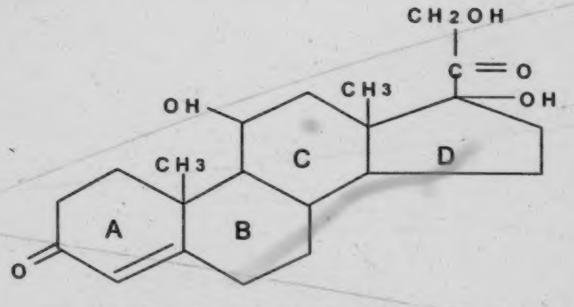
Figure 6. Carbon Skeleton



CARBON SKELETON

The other basic feature of glucocorticosteroids is the ketol grouping at position 20, oxygen atom on carbon atom 3, a double bond between carbon atom 4 and carbon atom 5, the methyl groups at carbon atom 18 and 19 and the hydroxyl group at carbon atom 11 [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980; Cope, 1972; Myles and Daly, 1974; Phillips, 1974; Dluhy et al., 1975; Applezweig, 1962; Sutherland,, 1970]. The two-carbon ketol side chain at carbon atom 17 is designated β, and the hydroxyl group at the same carbon atom is designated α [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980; Phillips, 1974; Bowman and Rand, 1980; Benet et al., 1984].

Figure 7. Hydrocortisone



CORTISOL (HYDROCORTISONE)

Cortisol's three-dimensional configuration resembles a chair, due the double between carbon atom 4 and carbon atom 5 [Haynes and Murad, 1985; Haynes and Larner, 1975]. These chemical characteristics determine the biological activity of corticosteroids.

According to their biological activity, the corticosteroids are classified as glucocorticosteroids and mineralocorticosteroids. If a corticosteroid has potency based on liver glycogen deposition, it is called a glucocorticosteroid. If one has potency based on sodium retention, it is called a mineralocorticosteroid. However, a corticosteroid may have both potencies. This biological activity is measured by the effects of glucocorticosteroids on the metabolism of carbohydrates, and on the kidney reabsorption of sodium [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980;

Laurence and Bennet, 1984; Cope, 1972; Myles and Daly, 1974; Phillips, 1974; Dluhy et al., 1975; Applezweig, 1962]. Topical anti-inflammatory and systemic glucocorticoid potencies are determined by the intracutaneous vasoconstriction and depression of plasma cortisol levels, respectively [Johanson et al., 1982]. Examples of the importance of chemical structure can be demonstrated: the high anti-inflammatory activity requires an α-hydroxyl group at C7 and a β-hydroxyl group at C11. Hormonal activity requires a double bond linking carbon atoms 4 and 5, and an oxygen atom on C3. Halogenation always increases anti-inflammatory and mineralocorticoid activity, but when associated to hydroxylation or methylation at C16, the process of adding halogen retains anti-inflammatory activity and reduces mineralocorticoid activity [Crossland, 1980]. Esterification in the 17a position increases glucocorticosteroid potency [Brattsand, 1990].

The possible corticosteroid isomers are derived from reduction of the ketone at C3: 3β-hydroxyl and 3α-hydroxyl; two are derived from saturation of the 4,5 double bond: 5α and 5β; and two derived from reduction of the ketone at C20. This forms an asymmetrical carbon in this point: α and β [Haynes and Murad, 1985; Haynes and Larner, 1975].

Most of the synthetic compounds are glucocorticoids with little or no mineralcorticoid activity. The following describes the changes in the chemical structure and the associated products:

- Introduction of a 1,2 double bound in the ring A: prednisone and prednisolone [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980; Aviado, 1972; Myles and Daly, 1974; Phillips, 1974; Dluhy et al., 1975; Applezweig, 1962; Cope, 1972; Sutherland, 1970; Benet et al., 1984; Brattsand, 1990];

- A methylation or fluoridation at position C6: methyl prednisolone and fluprednisolone [Crossland, 1980; Aviado, 1972; Phillips, 1974; Cope, 1972; Sutherland, 1970; Brattsand, 1990];
- Fluoridation at C9 associated to hydroxyl group: triamcinolone [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980; Aviado, 1972; Myles and Daly, 1974; Phillips, 1974; Sutherland, 1970; Brattsand, 1990];
- Fluoridation at C9 associated to methylation at C16: dexamethasone and betamethasone (is isomeric with dexamethasone, but its methyl group takes the β instead of the α configuration) [Haynes and Murad, 1985; Haynes. and Larner, 1975; Crossland, 1980; Aviado, 1972; Myles and Daly, 1974; Phillips, 1974; Dluhy et al., 1975; Sutherland, 1970; Brattsand, 1990];
- Fluoridation at C6 associated with methylation at C16: paramethasone [Crossland, 1980; Aviado, 1972; Myles, and Daly, 1974; Sutherland, 1970];
- Fluoridation at C9 and C6 associated with an hydroxyl group: fluocinolone (has mineral and glucocorticoid activity) [Crossland, 1980; Sutherland, 1970];
- Chlorination at C11 and C9, without substitution on C16: dichlorisone (has mineral and glucocorticoid activity)[Crossland, 1980; Sutherland, 1970];
- Substitution at the 17α ester position: beclomethasone dipropionate or budesonide [Brattsand, 1990; Greenberg et al., 1994; Johanson et al., 1982; Clissold et al., 1984; Procopiou et al., 2001].

3.5) Absorption

Glucocorticosteroid molecules enter cells by diffusion across their plasma membrane. This is supported, for example, by studies in tissue culture where the steroid concentrations required for binding to the glucocorticosteroid receptor were similar in either intact cells or in cell free homogenates [Haynes and Larner, 1975]. It is possible that steroids may be actively transported out of some cells. The extrusion process is temperature-dependent, saturable, glucose-dependent, and operates for only a few selected steroids [Gross et al., 1970]. A physiological role of the multidrug transporter P-glycoprotein (PGP) in the extrusion of cortisol is suggested specially for the more lipophilic steroids [Van Kalken et al., 1993].

Within the cytoplasm of the cell there is a specific glucocorticoid-binding protein which is the receptor for these steroids. These receptors exist in many tissues, since glucocorticosteroids have wide-spread effects throughout an organism [Haynes and Larner, 1975; Bamberg et al., 1996]. There are cytoplasmic receptors for steroids in most tissues of the young rat and rabbit [Ballard et al., 1974]. The glucocorticosteroid receptor of the rat liver has a molecular weight estimated to be 66 kd. However, the fibroblast receptor has a molecular weight of about 600 kd. This discrepancy, added to the demonstration that phospholipase decreases the apparent size of the receptor, suggests that the receptor may be a lipoprotein [Haynes and Larner, 1975; Hackney and Pratt, 1971].

Other proteins have been described in liver, kidney and other tissues such as the brain, which bind some glucocorticosteroids. In general, the proteins are found in the cytosol in higher concentration than the specific receptors, and they do not have a high affinity for the synthetic glucocorticosteroid. These cytosolic proteins can be distinguished from transcortin on the basis of their elution on DEAE columns, and by other types of protein fractionation techniques [Haynes and Larner, 1975].

The receptor-hormone complex undergoes a transformation that results in a change in its sedimentation coefficient. When this structural change (the exact nature is unknown) takes place, the receptor-steroid complex becomes capable of diffusing into the nucleus of the cell. Within the nucleus the receptor-steroid complex is bound to chromatin [Haynes and Larner, 1975].

The glucocorticoid receptors bind active glucocorticoid, proportional to their concentration and activity. The levels of plasma corticoid may be evaluated by quantifying their ability to compete with a radioactive glucocorticoid for binding to the specific receptor. The major advantage of this assay is that it can quantify levels of any of the synthetic glucocorticoids. There are occasional major differences in absorption and metabolism of the synthetic steroids [Haynes and Larner, 1975]. Cortisol and its synthetic analogous are effective when they are given orally [Haynes and Murad, 1985; Sutherland, 1970; Girdwood and Petrie, 1987]. Cortisol is readily and rapidly absorbed from gastrointestinal tract [Aviado, 1972]. Enteric coatings that are designed to reduce the incidence of dyspepsia delay absorption but do not reduce the total amount of cortisol which is absorbed. In general, the corticosteroids are absorbed in the upper jejunum, and peak concentrations in plasma occur in 30 min to 2h after their administration [Ehrich et al., 1992]. However, the desoxycorticosterone is ineffective when it is given orally [Haynes and Murad, 1985].

The water-soluble esters of cortisol and their synthetic congeners are given by the intravenous route, in order to rapidly achieve high concentrations in body fluids [Haynes and Murad, 1985]. Intramuscular suspensions of cortisol have prolonged effects [Haynes and Murad, 1985]. The glucocorticoids are absorbed from sites of local application: synovial spaces, conjuntival sac, skin, intestinal mucosa. The administration of topical glucocorticosteroids for long periods or for use in large areas can cause systemic effects [Haynes and Murad, 1985; Sutherland, 1970; Girdwood and Petrie, 1987].

3.6) Transport

More than 90% of cortisol is reversibly bound to plasma proteins [Haynes and Murad, 1985; Myles and Daly, 1974; Baxter, 1979; Bowman and Rand, 1980]. The responsible proteins are globulin, a glycoprotein, and albumin. The globulin is called transcortin or corticosteroid-binding-globulin (CBG) [Rocci et al., 1982]. A physiological role of the multidrug transporter P-glycoprotein (PGP) in the transport of cortisol out of the cell is suggested [Van Kalken et al., 1993]. CBG physiological levels are 3 to 5 mg/dl [Myles and Daly, 1974]. CBG increases in pregnancy, and decreases in patients with hepatic disease [Haynes and Murad, 1985; Myles and Daly, 1974; Bowman and Rand, 1980; Sutherland, 1970; Benet et al., 1984]. The molecular weight of CBG is about 52 kd; it can bind one cortisol molecule per molecule of protein. CBG binding capacity is 20 mg of cortisol per 100 ml of plasma. Its half life in the plasma is 5 days; and in electrophoresis CBG migrates as an α1-globulin [Myles and Daly, 1974; Ballard, 1979].

The Δ4-3-ketone is an essential chemical characteristic for binding to CBG. Many of the synthetic corticosteroids have substituent groups with the α configuration, particularly at C9 and C6, which will markedly interfere with binding to CBG. This feature of synthetic corticosteroids results in an increase in the concentration of free corticosteroid, and consequently an increase in its biological activity [Myles and Daly, 1974].

The CBG has high affinity but low total binding capacity for cortisol, whereas albumin has low affinity and high total binding capacity [Haynes and Murad, 1985; Myles and Daly, 1974; Baxter, 1979; Bowman and Rand, 1980; Girdwood and Petrie, 1987; Benet et al., 1984]. In situations of low or normal cortisol concentration, most cortisol is bound to globulin. Otherwise, in situations of high cortisol concentrations, the amount of cortisol bound to globulin increases, the free cortisol concentration rises, and the amount of cortisol bound to albumin changes little [Haynes and Murad, 1985; Myles and Daly, 1974; Baxter, 1979; Bowman and Rand, 1980].

The physiological concentration of albumin is about 4.0 g/dl of plasma [Myles and Daly, 1974]. In physiological conditions, the free cortisol or unbound plasma cortisol in physiological conditions is 0.1 to 1.8 mg/dl of plasma, and it accounts for the biological activity of the plasma [Myles and Daly, 1974; Baxter, 1979; Bowman and Rand, 1980; Benet et al., 1984).

3.7) Metabolism

The liver is responsible for 70% of corticosteroid metabolism [Haynes and Murad, 1985; Cope, 1972; Sutherland, 1970; Girdwood and Petrie, 1987; Hunter and Chasseaud, 1976; Johanson, 1982]. In humans, the intestinal sites of metabolism of cortisol and cortisone are saturated before the hepatic sites [Barr et al., 1984]. The glucocorticoid metabolism can occur by:

- Reduction of the 4,5 double bond in the liver and in extrahepatic sites. This reaction results in an inactive substance, called dihydrocortisol. In the second step dihydrocortisol is converted to tetrahydrocortisol [Haynes and Murad, 1985; Cope, 1972; Peterson and Pierce, 1960; Bowman and Rand, 1980];
- Reduction in the liver of the 3 ketone substituent to a 3-hydroxyl, to form tetrahydrocortisol. Tetrahydrocortisol is then converted to tetrahydrocortisone [Haynes and Murad 1985; Aviado, 1972; Cope, 1972; Peterson and Pierce, 1960; Bowman and Rand, 1980; Hunter and Chasseaud, 1976];
- Reversible oxidation of the 11-hydroxyl group occurs slowly in extrahepatic tissues, and rapidly in the liver [Haynes and Murad, 1985; Cope, 1972]. The corticosteroids with 11-ketone substitution require reduction to 11-hydroxyl group compounds for their biological activity [Haynes and Murad, 1985, Bowman and Rand, 1980; Sutherland, 1970; Benet et al., 1984];
- Reduction of the 20 ketone to a 20-hydroxyl which has little, if any, biological activity. This reduction forms cortol (α and β) and cortolone (α and β) [Haynes and Murad, 1985; Cope, 1972; Peterson and Pierce, 1960; Cope, 1972; Bowman and Rand, 1980; Sutherland, 1970]. The tetrahydrocortisol and tetrahydrocortisone represent 35% of the corticosteroid metabolites excreted, while the cortol and cortolone represent 30% of the same metabolites [Peterson and Pierce, 1960].
- Oxidation of the 17-hydroxyl group to form 17-ketosteroids and a two-carbon fragment. The 17-ketosteroid lacks in corticosteroid activity, but has weak androgenic properties [Haynes and Murad, 1985, Bowman and Rand, 1980; Sutherland, 1970);

There are liver enzymes capable of carrying out the reduction of the ketone group: one is in the soluble fractions (5β-isomer) and another in microsomes (5α-isomer) [Aviado, 1972; Cope , 1972]. These enzymes require NADPH as a hydrogen donor [Cope, 1972]. There are other enzymes responsible for the reduction of Δ1 unsaturation of prednisone, 2-hydroxylation, and 20-hydroxylation [Cope, 1972]. Most of these enzymes are part of the cytochrome P-450 system, that can easily inactivate glucocorticosteroids with lipophilic groups such as 17α-valerate and 17α,21-dipropionate [Brattsand, 1990]. Regioselective and stereospecific hydroxylation of these molecules in positions 2,6,7,15,16, and 21 has been studied in detail, and has been shown to be catalysed by several forms of cytochrome P450 from families 1, 2, and 3 in rats and from families 3 and 4 in humans [Pichard et al., 1991]. Dexamethasone and prednisone are inducers of cytochrome P450 3A, increasing the accumulation of mRNA and protein [Pichard et al., 1991]. The locally-acting steroid budesonide is metabolized by cytochrome P450 3A enzymes in human liver [Jonsson G. et al., 1995].

Most of the ring-A-reduced metabolites are coupled through 3-hydroxyl in the liver or kidney with sulphate or glucuronic acid, to form water soluble sulphate esters or glucuronides. These compounds can be excreted in the urine [Haynes and Murad, 1985; Cope, 1972; Bowman and Rand, 1980; Ehrich et al., 1992; Greenberg, 1994]. Excretion is largely dependent on prior conjugation of the steroids with glucuronic acid [Peterson and Pierce, 1960]. Glucuronyl transferase catalyses glucuronidation of various endogenous substances [Sutherland, 1970].

Corticosteroid use is considered relatively safe during pregnancy due to a placenta enzyme, 11β-OH-dehydrogenase. This enzyme inactivates corticosteroids, and leads to low concentration of active drug in the fetus [Ehrich et al., 1992].

Glucocorticosteroids with 16α, 17α acetyl groups such as budesonide, have topical anti-inflammatory activity, stability in extra-hepatic tissues (airways and intestinal walls), and about 90% first pass hepatic metabolism to products which posses minimal or no biological activity [Greenberg, 1994; Brattsand, 1990].

The majority of corticosteroids are excreted in the urine. [Haynes and Murad, 1985; Aviado, 1972; Fukushima et al, 1960; Cope, 1972]. Corticosteroids are detectable in small amounts in fecal, biliary and CO_2 excretion [Haynes and Murad, 1985; Haynes and Larner, 1975; Sutherland, 1970].

The cortisol plasma half-life is about 1.5 h, and the half-life of prednisolone is 1 to 2 h. However, half life of the biological effect of prednisone is in the range of approximately 24h [Haynes and Murad, 1985; Cope, 1972; Sutherland, 1970]. The corticosteroid metabolism is greatly slowed by a 1,2 double bond, and by fluoridation. These chemical features prolong its half-life [Haynes and Murad, 1985; Cope, 1972].

The physiological human plasma cortisol level in the morning is 5-20 mg/ml, and in the afternoon is 2.5-10 mg/ml [Greenspan and Baxter, 1994; Griffin and Wilson, 1992; Jonetz-Menztel and Wiedemann, 1993]. In rats and mice, the plasma corticosterone levels vary greatly according to the technique and sample collection methods. Generally, using high performance liquid chromatography, a baseline range of 50 to 300 ng/ml and 50 to 400 ng/ml can be expected for mice and rats respectively depending on the time of day the sample is taken [Shimizu et al., 1983]. In both mice and rats the highest concentrations of plasma corticosterone are observed between 4:00 pm and 10:00 pm [Coffigny et al., 1978].

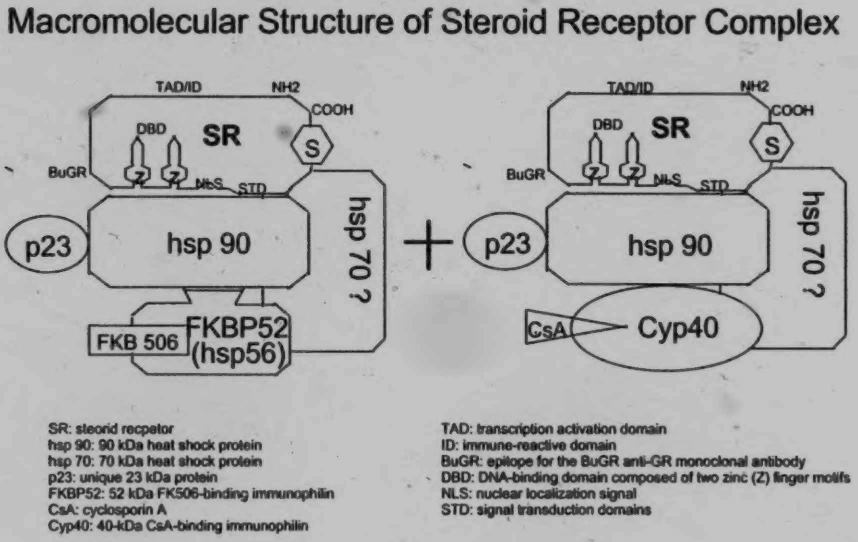
3.8) Mechanism of Action

Glucocorticosteroid molecules enter cells by diffusion across their plasma membrane [Haynes and Larner, 1975; Sandborn and Faubion, 2000]. The action of steroids does not require cytosolic kinases [Alberts et al., 1994]. Within the cytoplasm there is a specific glucocorticoid-binding protein which is considered to be the receptor for these steroids. These receptors exist in many tissues, and they constitute the intracellular receptor superfamily or steroid-hormone receptor superfamily, as well as those receptors related to thyroid hormones, retinoids and to vitamin D [Haynes and Larner, 1975; Alberts et al., 1994; Schulman et al., 1994; Sheppard et al., 1999]. The receptor-hormone complex undergoes a transformation that results in its activation. The activation of this receptor consists of binding of ligand to the receptor, which dissociates an inhibitory protein complex. This results in the exposition of the DNA-binding site of the receptor [Alberts et al., 1994]. When this structural change takes place, the receptor-steroid complex becomes capable of diffusing into the nucleus of the cell through the nuclear pores. Within the nucleus, the complex is bound to the nuclear chromatin [Haynes and Larner, 1975; Bamberger et al., 1996]. This reaction influences the synthesis of specific mRNA and consequently protein synthesis, which is ultimately responsible for the glucocorticoid response [Baxter, 1979; Bamberger et al., 1996]. The cell response to glucocorticosteroids involves two steps: 1) an early primary response, which consists of the activation of primary-response genes by steroid hormone-receptor complexes, and consequently induction of synthesis of primary-response proteins; and 2) a delayed secondary response which involves activation of secondary-response genes by the primary-response proteins. These secondary-response proteins also shut off the primary-response genes [Alberts et al., 1994].

A model of the macromolecular structure of inactivated or untransformed steroid receptor (SR) complex is presented in Figure 7. The SR complex has one molecule of hormone binding receptor [Denis and Gustafsson, 1987] with the indicated functional domains: transcription activation domain (TAD), immune-reactive domain (ID), DNA-binding domain (DBD), nuclear localization signal (NLS) and signal transduction domain (STD). In addition, the complex also has two molecules of heat shock protein (hsp) 90, and unknown numbers of FKBP52, cyclophilin-40 and p23 molecules [Denis and Gustafsson, 1987; Tai et al., 1986; Owens-Grillo et al., 1995; Smith et al., 1990; Bresnick et al., 1990; Sandborn and Faubion, 2000]. Another heat shock protein, hsp 70 is also included in the model [Srivinasan et al., 1994]. The binding of hsp 90 to the steroid receptor as indicated occurs in the domain named STD [Housley et al, 1990]. The binding of p23, a co-chaperone to the complex occurs through hsp 90, as well as the binding of FKBP52 and Cyp40 to the steroid receptor [Jhonson and Toft, 1995; Lebeau et al., 1992; Renoir et al., 1992]. Two distinct SR complexes may exist, each distinguishable by the presence of FKBP52 or Cyp40 subunits as is the case of the mouse glucocorticosteroid receptor. These two distinct receptors may be functionally different, and one may be the precursor of the other [Owens-Grillo et al., 1995]. These two different SR isoforms are generated by alternative splicing of the human SR pre-mRNA, and are termed SRα and Srβ [Bamberger et al., 1996]. The distinction between SRα and Srβ may be more relevant than previously thought. In fact, overexpression of SRβ antagonizes the effects of hormone activated SRα on a glucocorticoid-responsive gene [Bamberger et al., 1995; Honda et al., 2000]. This finding would explain why we observe positive effects of glucocorticosteroids on gene expression in certain conditions, and negative effects in other conditions.

Figure 8. Steroid Receptor

(From webpage: http://www.mco.edu/depts/pharm/ssrc.html)


Macromolecular Structure of Steroid Receptor Complex
TAD/ID
NH2
COOH
DBD
SR
S
BuGR
Z
Z
NLS
STD
p23
hsp 90
hsp 70 ?
FKB 506
FKBP52
(hsp56)
+
TAD/ID
NH2
COOH
DBD
SR
S
BuGR
Z
Z
NLS
STD
p23
hsp 90
hsp 70 ?
CsA
Cyp40
SR: steorid recpator
hsp 90: 90 kDa heat shock protein
hsp 70: 70 kDa heat shock protein
p23: unique 23 kDa protein
FKBP52: 52 kDa FK506-binding immunophilin
CsA: cyclosporin A
Cyp40: 40-kDa CsA-binding immunophilin
TAD: transcription activation domain
ID: immune-reactive domain
BuGR: epitope for the BuGR anti-GR monoclonal antibody
DBD: DNA-binding domain composed of two zinc (Z) finger motifs
NLS: nuclear localization signal
STD: signal transduction domains

Most tissues are direct targets for glucocorticosteroids, and all tissues may be influenced indirectly by this steroid. The physiological responses are diverse [Baxter, 1979]. The glucocorticosteroid response depends on the nature of the particular steroid, and on the target tissue. The various types of steroids are separated according to their activity in four classes [Samuels and Tomkins, 1970]:

- Optimally active steroids: when the steroids are present in a concentration sufficient to saturate the receptors, and elicit the maximal glucocorticosteroid response;
- Suboptimal steroids: when the steroids are not present in a concentration sufficient to saturate the receptors, and elicit the intermediate glucocorticosteroid response;
- Anti-inducers steroids: when the steroids do not elicit a glucocorticosteroid response;
- Inactive steroids: when the steroids neither bind to receptor nor influence glucocorticosteroid response.

If one compares the biological activities of the steroids with suboptimal and antiglucocorticoid activity in different tissues studies, considerable variation is seen. One reason for this is that metabolism may convert an inactive glucocorticoid to an active one [Samuels and Tomkins, 1970] .

After exposure of cultured hepatoma cells to dexamethasone, about 70% of the total cytoplasmic receptor localizes in the nucleus [Rousseau, 1973]. This suggests that the function of the glucocorticoid is to influence the nuclear localization of the glucocorticoid-receptor complexes. In hepatoma cells, there is a rapid disappearance of the glucocorticosteroid response upon removal of the inducer steroid. Consequently, there is a rapid dissociation of steroid from the receptors, which is associated with a disappearance of receptors from the nucleus, and their reappearance in the cytosol. These findings suggest that in the induced state, the cytoplasmic receptor remains in the nucleus, stoichiometrically combined with the corticosteroid [Samuels and Tomkins, 1970; Rousseau, 1973]. The level of glucocorticoid receptor mRNA in a number of target tissues and in many different cells is regulated by the presence of glucocorticosteroids, and this SR mRNA is different according to the steroid half-life [Shimojo et al., 1995].

The activation of the SR complex is favored by heating, dilution, salt treatment or by divalent cations. "Activation" is the change that must occur in the SR complex following binding of steroid to receptor and prior to nuclear binding. This activation is a temperature-dependent step. Cytoplasmic fractions were exposed to salt, then chilled, and the salt was removed; this resulted in SR complexes binding to the nuclei. The nature of this change is not understood. In some systems divalent cations may influence the activation of the SR complex [Baxter, 1979].

As cytosolic binding increases, nuclear binding also increases, and does not appear to level off [Willian and Gorski, 1972]. Thus, the nuclear acceptor capacity appears to exceed the cytosol receptor capacity. Nuclear sites are highly specific in relation to the type of cytoplasmic receptor, and to the tissue of origin. The nuclear acceptors are localized on the chromatin, which binds the glucocorticoid-receptor complexes [Baxter, 1979]. SR complexes bind to glucocorticosteroid responsive elements as a dimer, which is thought to be the active regulatory form [Chalepaski et al., 1990; Drouin et al., 1992]. A nuclear corticosterone binding site has been identified in enterocytes that differs from other glucocorticosteroid receptors [Sheppard et al., 2000]. Further work is necessary to identify the possible physiological and pathological importance of these receptors.

In order to activate a gene, an intracellular receptor needs the presence of the right combination of other gene regulatory proteins. Thus, each steroid has a specific response, and each cell with receptors has a different combination of other gene regulatory proteins

[Johnson, 1987]. Since chromatin is a complex of DNA and associated proteins, the acceptors are in one and/or both these components. Two lines of evidence suggest a role for DNA in acceptor activity. First, the glucocorticosteroid-receptor complexes bind to purified DNA, and the capacity of DNA for binding to the complexes is even larger than that of chromatin. Second, the loss of nuclear binding capacity parallels the DNA loss caused by treatment with deoxyribonuclease (DNAase). DNA binding is much more sensitive to ionic strength and to divalent cations than is nuclear binding [Baxter, 1979; Tomkins et al., 1972].

In general, the glucocorticosteroid response requires RNA synthesis, and subsequently protein synthesis. The factor that accumulates in response to the glucocorticoids is the mRNA for the enzyme in question. This occurs for example with trehalase mRNA and trehalase, a glycoprotein of the brush border membrane of the enterocyte and kidney proximal tubule epithelial cells [Galand et al., 1995]. Furthermore, the glucocorticoid effect is blocked by inhibitors of RNA synthesis, and the number of polysomes involved in enzyme synthesis are increased in response to corticosteroid. However, in some situations the response induced by glucocorticosteroid may be indirect. Initially, this response may involve only protein synthesis and consequently other pathways and functions related to this protein [Baxter, 1979].

There is a repressor of tyrosine aminotransferase mRNA which inhibits the translation of the mRNA. This repressor is labile, and when RNA synthesis is inhibited the repressor disappears. It is postulated that the glucocorticoid-receptor complex inhibits the synthesis of this "repressor", resulting in an accumulation of mRNA. However, the interaction of the glucocorticosteroids with its nuclear receptor results in the induction of either positive events (transactivation), or negative events (transrepression) by repression of gene transcription and/or alteration of mRNA half-lives [Krane, 1993; Sakai et al., 1988]. Repression of transcription is achieved by inhibition of various transcription factors such as activator protein-1 (AP-1), nuclear factor κ B (NFκB) (by inducing the expression of an inhibitor of NFκB called I-κB), and the signal transduction and activatiors of transcription family (STAT) [Barnes, 1998; Rosene et al., 1998; Birt et al., 1999; Rogler et al., 1999; Sandborn and Faubion, 2000].

Glucocorticoid receptors function as a necessary and sufficient factor for the transcriptional activation of the MTV-CAT (mouse mammary tumor virus as measured by chloranphenicol acetyl transferase assays) fusion gene [Giguere et al., 1986]. The magnitude of this induction reveals that glucocorticoid receptors may act as a transcriptional "switch", which may involve a silent promoter containing a glucocorticoid response element to an active state. This stimulation is totally dependent upon the presence of glucocorticoid hormones. The mechanism involved in the activation of glucocorticoid-receptor is poorly understood, but it is likely to involve allosteric transitions within the protein [Giguere et al., 1986].

Godowsaki and colleagues (1987) proposed the derepression mechanism, by which glucocorticoid hormone binding might yield the functional enhancer-activating protein. Ligand-stimulated structural transition may unmask DNA-binding or enhancer-activation domains already folded in their functional configuration. This derepression mechanism predicts that constitutive enhancer activating proteins, functional even in the absence of hormone, might appear upon deletion of a region that masks or represses otherwise functional or enhancer-activation domains [Godowsaki, 1987].

There are special situations where unresponsiveness or hyper-responsiveness occurs to glucocorticoids. In the course of the treatment of childhood lymphoblastic leukemia, unresponsiveness to glucocorticosteroid, may occur [Klumper et al., 1995], possibly due to a decrease in the amount of cytoplasmic receptors. This response is not influenced by the hormone, and is increased by mutagens [Sibley and Tomkins, 1974]. Thus, a mutational event may be involved in unresponsiveness to glucocorticoid. Although the receptor defect appears to be the most common, other steps in glucocorticoid action are also blocked because a mutational event interferes with steps in corticosteroid action. However, defects in cellular entry and steroid metabolism have not been found [Sibley and Tomkins, 1974]. In contrast, defects in the receptor itself, in the ability of the receptor-glucocorticosteroid complex to bind to the nucleus (whereby the steroid receptor complex binds more tightly to the nucleus) have been found [Sibley and Tomkins, 1974; Yamamoto et al., 1974; Stratakis et al., 1994]. In other cases of unresponsiveness to glucocorticosteroids, normal steroid binding with the receptor and nuclear binding have been demonstrated. It was suggested that the mechanism of unresponsiveness is localized in some site distal to the initial steroid-receptor interaction [Lippman and Thompson, 1974].

Glucocorticoid resistance can emerge through defective nuclear binding and/or mutation in the receptor as indicated by a slower sedimentation in density gradients, as well as through blocks in mechanisms distal to nuclear binding of the complex. Several steroid resistant lines appear to be a mutation in the receptor as indicated by a slower sedimentation in density gradients [Sibley and Tomkins, 1974; Yamamoto et al., 1974; Schottelius et al., 2000]. Therefore, the lack of responsiveness to glucocorticosteroids may be genetically determined.

Some patients with colitis do not respond to high doses of corticosteroids, and these persons have a low glucocorticoid receptor content in their mononuclear cells [Madrestma et al., 1995]. Interestingly, antibodies to lipocortin-1 do not have a role in the development of steroid-resistance in children with inflammatory bowel disease [Beattie et al, 1995]. In contrast, hyper-responsiveness to steroids occurs in patients with open-angle glaucoma, and in persons homozygous for the postulated gene defect which causes this disease [Baxter, 1979; Tripathi et al., 1992].

Other mechanisms that could explain the resistance to glucocorticosteroids involve the induction of multidrug resistance-1gene (MDR-1), that results in the increased expression of the membrane-based drug efflux pump pump P-glycoprotein [Nakayama et al., 1999; Sandborn and Faubion, 2001].

The tendency of glucocorticoids to produce hyperglycemia and carbohydrate intolerance follows from the inhibition of glucose uptake, tissue catabolism, and conversion of substrate to glucose. Other hormones can also influence these reactions [Baxter, 1979; Sutherland , 1970; Girdwood and Petrie, 1987; Wise et al., 1973; Weber et al., 1965; Pagano et al., 1983]. The increase in the tissue catabolism results in the release of fatty acids, glycerol and nucleic acids. These substrates are used for gluconeogenesis. The mechanism of glucocorticoid-induced gluconeogenesis has been postulated to be explained by the increased precursor amino acids and/or the induction of gluconeogenic pathways in the liver. The steroid stimulation of gluconeogenesis in the liver may be blocked by inhibitors of protein or RNA synthesis [Exton, 1972; Weber et al.,1965]. The free fatty acids (FFA) arising from lipolysis may spare glucose and amino acids from being utilized for energy production. This can save glucose and amino acids, the most important substrates for gluconeogenesis and for enzyme production. FFA provide an energy source and influence gluconeogenesis through the production of NADH. However, these possibilities are controversial: while FFA can increase gluconeogenesis from a variety of substrates in liver slices and in the perfused liver, there is no stimulation of gluconeogenesis when FFA are added in a physiologic manner. Thus, further studies *in vivo*, specially in humans, are required to establish whether or not FFA are important in gluconeogenesis [Exton, 1972].

The major site of gluconeogenesis is the liver, but renal gluconeogenesis can be important, especially in the fasting state [Exton, 1972]. The mechanisms involved in the increase of gluconeogenesis are the increase in the hepatic level of glycogen synthetase, and secondary hyperinsulinism (insulin is a potent activator of the glycogen synthetase) [Baxter, 1979; Haynes . and Murad, 1985; Haynes and Larner, 1975; Girdwood and Petrie, 1987].

If the actions of other hormones are not observed unless there has been exposure to glucocorticosteroid, this action is termed "permissive". This occurs in the sensitivity of the fat cell to epinephrine-induced lipolysis. Many of these permissive actions are associated with influences on hormones that stimulate adenyl cyclase [Baxter, 1979; Haynes and Murad, 1985; Haynes and Larner, 1975]. The glucocorticoid actions on fat cells result in the development of the Cushingoid appearance (redistribution of fat, with an accumulation in the face and trunk and wasting in the extremities). Steroids increase lipolysis and decrease glucose uptake and metabolism in fat cells. It is likely that fat tissues have varied hormonal sensitivity, such that glucocorticosteroid effects are dominant in the extremities, and insulin actions are more effective in the trunk [Baxter, 1979; Sutherland, 1970].

Glucocorticosteroid administration increases the FFA in plasma. The action of glucocorticosteroids on increasing fatty acid release apparently results 1) from inhibition of glucose metabolism, with concomitant decrease in glycerol production; and 2) from potentiation of the actions of a wide variety of lipolytic hormones including cathecolamines, thyroid hormones, growth hormone and ACTH [Baxter, 1979].

Glucocorticosteroids have also been reported to have a specific effect on plasma lipoproteins. Corticotropin administration resulted in a rapid decrease in the concentrations of plasma polipoprotein B, low- density lipoprotein (LDL), cholesterol and triglyceride. In contrast, dexamethasone treatment did not change any of the apo B-containing lipoproteins. High-density lipoproteins (HDL) increased with both treatments, and apo A-I increased only with dexamethasone. Up-regulation of low-density lipoprotein receptor activity *in vitro* is equally observed after incubation with corticotropin. Therefore, glucocorticosteroids have a direct effect on lipoprotein metabolism, primarily on apo B-containing lipoproteins [Berg and Nilsson-Ehle, 1994]. Knowing that the lipoproteins are responsible for the transport of the re-synthesized lipids to the blood or lymphatic system, if we had a negative effect on the synthesis of lipoproteins as observed with glucocorticosteroids we would expect alterations in the lipid uptake and\or accumulation of lipids at the enterocyte level.

The glucocorticoid catabolic actions are mainly seen in lymphoid cells, muscle, skin, fat cells and fibroblastic tissues. The steroid catabolic effect is not generally seen in liver, gut, kidney, brain, heart or blood cells. It is likely that this catabolic or anti-anabolic effect is due to the induction of proteins which are ultimately toxic for the cell [Baxter, 1979].

3.9) Liver

The liver is one of the few targets for glucocorticosteroids in which there is anabolism. However, some functions are inhibited; for example, DNA synthesis [Loeb et al., 1973]. This glucocorticoid action in the liver involves gluconeogenesis.

3.10) Muscle

Muscle is the target tissue where the glucocorticoid actions are catabolic, and is responsible for the negative nitrogen balance observed in patients with Cushing's syndrome. This steroid myopathy is associated with elevations in the concentration of muscle enzymes in the plasma [Ehrich et al., 1992; Baxter, 1979; Kusunoki et al., 1992].

3.11) Immunology and Inflammatory Responses

Glucocorticoids are the most potent drugs for treating a number of diseases in which abnormal immunological reactions are thought to be a major cause of the pathology. The catabolic actions of the corticosteroid on mast cells, lymphoid cells, macrophages, other blood elements, endothelial cells and fibroblastic tissues form the major basis for immunological and inflammatory suppressive responses [Ehrich et al., 1992; Baxter, 1979; Sutherland, 1970; Klebl et al., 1994]. Glucocorticosteroids affect different stages of the immunological response: antigen processing and penetration of basement membranes by antigen-antibody complexes; actions on macrophage function;

delayed hypersensitivity reaction; T-cell mediated and spontaneous cytoxicity; immunoglobulin levels without interference in specific antibody production; reticuloendothelial clearance of antibody-coated cells; cellular helper cell functions; inflammatory reactions; and vascular reactions [Ehrich et al., 1992; Baxter, 1979; Cupps and Facui, 1982; Gillis et al., 1979; Arya, 1984; Wu et al., 1991; Bowen and Faunci, 1984; Goulding and Guyre, 1993].

The inflammatory reactions involved with glucocorticoid response are: an increase in circulating neutrophils; a decrease in circulating lymphocytes and monocytes; inhibition of the accumulation of neutrophils and macrophages at inflammatory sites (may be the most important factor); a reduction in edema formation; and a reduction in the increase in vascular permeability. Some of these effects are mediated by interfering with the synthesis or release of prostaglandins, leukotrienes and bradykinins; complement activation and action; margination and sticking of leukocytes to vascular endothelium; membrane permeability; and the action of migration inhibition factor (MIF) which causes macrophages to adhere to the endothelium [Ehrich et al., 1992; Baxter, 1979; Haynes and Murad F, 1985; Haynes and Larner, 1975; Skubitz et al., 1981; Coates et al., 1983; Blackwood et al., 1982; Castagnulo et al., 2001].

Inflammatory cytokines have selective and specific inhibitory effects on the expression of the brush border hydrolase sucrase-isomaltase in *vitro* and in *vivo*, providing evidence for a previously unrecognized mechanism for disaccharidase deficiency in intestinal inflammation. The most well characterized cytokines involved in this process include IL-6, IL-1α ,IL-1β, TNF-a and IFN-γ [Ziambaras et al., 1995]. This finding brings up the question whether glucocorticosteroids inhibiting the production, expression and release of cytokines, enhance the expression and activity of other brush border membrane hydrolases. For example, budesonide has been described to have a negative effect on the expression of IL-3 and IL-5 [Lantero et al, 1996], but both budesonide and methyprednisolone do not affect the expression of IL-2 [Elitsur et al., 1998].

3.12) Healing and Scar Tissue

Glucocorticoids exert a negative influence on wound healing when given systemically or topically, due to a decrease in collagen formation and in the fibroblast response [Baxter, 1979; Sutherland, 1970].

3.13) Blood Cells

Glucocorticoids increase the plasma hemoglobin concentration by about 15%. The marrow response to glucocorticoid is normoblastic, and it can sometimes mask a megaloblastic anemia. The blood eosinophils and basophils are depressed in Cushing's disease or after glucocorticoid therapy. The acute response to giving steroids is an increase in thrombocytes, but after chronic administration there is thrombocytopenia. The polymorphonuclear cells increase and mononuclear cells decrease (70% in circulating lymphocytes, and 90% in monocytes). Although the neutrophil number increases, their phagocytic ability actually decreases [Baxter, 1979; Sutherland, 1970; Girdwood and Petrie, 1987; Haynes and Murad, 1985; Haynes and Larner, 1975]. This increase in neutrophil number is due to the glucocorticoid's protective effect on human neutrophil survival by delaying apoptosis [Liles et al., 1995].

3.14) Lung

Glucocorticosteroids improve severe bronchial asthma by enhancing the β adrenergic responses (permissive action) that result in bronchodilatation [Baxter, 1979]. Glucocorticosteroids induce the production of surfactant in the lung of the fetus, and accelerate pulmonary maturation, thereby preventing the respiratory distress syndrome. [Avery, 1995; Gonzales et al., 1994; Boggaram et al., 1991; Delemos, 1970; Motoyama, 1971].

3.15) Cardiovascular System

The heart contains glucocorticoid receptors, and some persons given steroids have an increase in cardiac output, with a decrease in peripheral resistance [Sambhi, 1965; Haynes and Murad, 1985; Haynes and Larner, 1975; Ballard et al., 1974].

Patients with spontaneous Cushing's syndrome and hypertension have been found to have elevations of plasma renin substrate, the protein which acts to release angiotensin. Furthermore, increased vascular reactivity to infused pressor substances has been observed in patient with Cushing's syndrome, and after high doses of glucocorticosteroid given to normal subjects [Krakoff et al., 1975; Sutherland, 1970; Kusunoki et al., 1992]. This steroid-induced hypertension is not associated with steroid-induced hyperinsulinemia [Whitworth et al., 1994].

Electrocardiographic changes associated to hypokalemia have been reported [David et al., 1970], due to the glucocorticoid action influencing in serum electrolytes such as potassium and calcium. Other changes such as cardiac conduction alterations and even arrhythmia were described after glucocorticoid administration. These can be explained by hypokalemia and/or enhancement of β adrenergic stimuli [Shimidt, 1972].

3.16) Endocrine System

The glucocorticosteroids have some actions on the metabolism of the thyroid hormones, decreasing uptake, clearance rate, and turnover of ^{131}I. These effects are reversible by the administration of thyroid stimulating hormone (TSH). The mechanism that causes this effect is probably at the pituitary level, decreasing the thyrotropin-releasing factor (TRF) from the hypothalamus. However, with continued glucocorticoid administration there occurs almost always an escape from the suppression of serum TSH concentrations. This results in a normal thyroxine concentration in plasma. Otherwise, thyroid hormone accelerates the metabolism of corticosteroids [Baxter, 1979; David et al., 1970].

The glucocorticosteroid actions appear to be generally antagonistic to growth hormone. This is likely due to the fact that a major mechanism of growth hormone action is to influence the production of somatomedin, which may have insulin-like actions. Otherwise, growth hormone decreases the uptake of glucose in muscle and enhances lipolysis in adipose tissue. These latter actions of growth hormone are parallel to those of the glucocorticoids [Baxter, 1979; David et al., 1970].

In general, glucocorticoid tends to depress the serum calcium, whereas parathyroid hormone tends to elevate it [Baxter, 1979].

3.17) Fluid and Electrolyte Balance

The glucocorticoids influence salt and water balance by their mineralocorticoid actions [Sabatini et al., 1993]. For example, dexamethasone and prednisolone have very weak sodium-retaining properties. The corticosteroids also influence fluid balance by increasing the glomerular filtration rate and renal plasma flow, and antagonizing the release of antidiuretic hormone (ADH) and/or exerting an anti-ADH-like action. These latter effects are more noticeable in the Addisonian patient who is treated with only aldosterone or desoxycorticosterone [Sutherland, 1970; Baxter, 1979].

3.18) Bone and Calcium Metabolism

The action of glucocorticoids on bone and calcium metabolism is the major limitation to long-term therapy. The glucocorticosteroid-induced osteoporosis tends to be severe and debilitating, with damaging changes in bone persisting even after cessation of glucocorticoid therapy. It is likely that glucocorticoid-induced catabolism in the matrix of bone is responsible for this bone damage [Ehrich, 1992; Sutherland, 1970; Baxter, 1979; Madsen and Andersen, 1994; Kasperk et al., 1995].

Corticosteroids promote hypocalcemia due to a steroidal alteration in the distribution of calcium between the extracellular and intracellular compartments, and due to a decrease in the intestinal absorption and renal tubular reabsorption of calcium [Baxter, 1979; Krane, 1993].

The regular measurement of bone density in persons taking steroids may be helpful in identifying persons at risk for the development of osteoporosis [Zelissen et al., 1994].

3.19) Growth

Corticosteroids have catabolic and anti-anabolic effects on connective tissue and muscle, suppress growth hormone secretion, and thereby limit the use of corticosteroids in children, because of their suppression on growth [Baxter, 1979; Kusunoki et al., 1992].

In rats, the administration of corticosteroids as cortisone acetate classically impairs growth, a finding that is reflected in baby weight gain. Furthermore, this classical growth-impairing effect is more pronounced in older (26 days) than in the younger (21 days) animals [Henning and Leeper, 1982].

3.20) Central Nervous System

Psychiatric disturbances are associated with the use of glucocorticosteroids. Depression is the most common manifestation of Cushing's, a syndrome caused by steroid adverse effects, whereas elevation of mood or frank euphoria is common in the iatrogenic disease caused by the intake of steroids [Ehrich et al., 1992; Sutherland, 1970; Kusunoki et al., 1992] . The moods changes are reversible with reduction in dose or discontinuation of therapy. The perception of taste, smell and sound stimuli are elevated in hypercorticism [Haynes and Murad, 1985; Haynes and Larner, 1975]. Benign intracranial hypertension is a rare condition with unknown etiology, although steroid use has been implicated [Newton and Cooper, 1994].

3.21) Gastrointestinal Tract

Cushing's syndrome has been implicated as causing an increased incidence of peptic ulceration [Nicoloff, 1969; Okabe et al., 1971; Cooke, 1973]. It is suggested that the glucocorticoids increase acid secretion. It is likely that the corticosteroids have catabolic effects in the stomach, and this could explain the mechanism for ulceration in animals [Ehrich et al., 1992; Baxter, 1979, Kusunoki et al., 1992; Kanemasa et al., 1999]. A steroid reduction on the level of prostaglandins in the gastric mucosa is another factor that explains the corticosteroid induced ulceration [Orlicz-Scczesna et al., 1994; Avunduk et al., 1992]. Ulceration risk doubles with steroid therapy [Messer et al., 1983; Ponce et al., 1991; Rohrer et al., 1999, Maruyama et al.,1999], and delayed healing of chronic gastric ulcers has been observed [Kuwayama et al., 1991]. This delay in healing can be prevented by supplementation of polyunsaturated fatty acids demonstrating interaction between steroids and dietary lipids [Manjari and Das, 2000]. Other gastrointestinal effect described in humans is the increase of appetite [Drug Evaluations Annual, 1991]. In contrast, in rats no significant differences in food intake have been described [Henning and Leeper, 1982].

The following sections will be give more details about glucocorticoid action on the small intestine.

3.22) Effects of Glucocorticosteroids on Intestine

In rats, the most commonly used animal model for the description of the effects of steroids on the intestine, corticosteroids classically reduce the body weight gain of these animals. No alterations in the food intake of animals have been described [Henning and Leeper, 1982].

Significant role for glucocorticosteroids on the trophic status of the intestine has been suggested by the finding that adrenalectomy in Sprague-Dawley rats results in atrophy and disorganization of the epithelium as well as increases in villus apoptosis and reduction in crypt cell proliferation [Foligne etal., 2001].

3.22.a) Effects of Glucocorticosteroids on Intestinal Uptake

The administration of prednisolone for seven days in adult rats has little effect on the intestinal mucosal structure or cell kinetics, but does enhance the absorptive capacities of the jejunum and ileum for galactose, when measured by a recirculation-perfusion technique [Batt and Peters, 1976; Batt and Peters, 1975]. This is due to an increase in the value of the maximal transport rate (V_{max}) of the transporter, without alterations in the value of the apparent affinity constant (K_m) [Scott et al., 1980; Batt and

Scott, 1982]. There is also steroid-enhanced activity of the intestinal brush border membrane enzyme activities, such as α-glucosidase, naphthylamidase and β-glucosidase [Scott et al., 1980]. Steroids increase the rate of synthesis of brush border membrane proteins, associated with an increased glycoprotein content of this membrane [Scott et al., 1981], and an increase in the membrane-bound ribosomal RNA (mRNA) content of the enterocytes. This leads to the enhanced synthesis of membrane proteins associated with a likely proliferation of the rough endoplasmic reticulum [Batt and Scott, 1982]. Short-term prednisolone dosing enhances the ileal epithelial cell migration rate, and decreases cell turnover time [Batt and Scott, 1982]. Also, the basolateral membrane enzyme activities and mitochondrial enzymes in rats are enhanced with steroids; however, the lysosomal or peroxisomal enzyme activities do not change [Scott et al., 1980]. Administration of prednisolone in rats for longer intervals (28 days) sustains these short-term stimulatory effects on intestinal absorptive and digestive functions, but long-term prednisolone is associated eventually with a small decrease in the height of the villus [Murphy, 1982].

In contrast, the administration of betamethasone-17-valerate (a locally rather than a systematically active glucocorticosteroid) decreases intestinal crypt cell turnover, decreases villous height and crypt depth, decreases the epithelial cell migration rate, and increases the transit time of enterocytes along the villus [Murphy, 1982]. Absorption per enterocyte is increased, but betamethasone reduces the total number of enterocytes, and hence there is no net change in the absorption per centimeter length of intestine. The predominant activity of this steroid may be the stimulation of enterocyte function or the reduction of the enterocyte population.

Following intestinal resection in rats, the short-term administration of prednisolone increases the adaptive hyperplasia in the remaining ileum, with an increase in villous height and in crypt depth, as well as an enhancement of the epithelial cell migration rate and of the villous transit time. As well, prednisolone increases enzyme activity per centimeter length of intestine, without changes in the enzyme activity in individual enterocytes. Higher doses of prednisolone result in only slight villous atrophy. In contrast, more prominent morphological changes may result from the administration of long-acting glucocorticoids, as they have a more marked effect on DNA metabolism and decreased crypt cell proliferation [Batt and Scott, 1982].

Dexamethasone (128 mg/ kg / day given subcutaneously [SC] for 7 days), dropped the weight, protein and DNA content of the duodenojejunal and ileal mucosa of sham operated rats, as well as in rats which underwent an 80% jejunoileal resection [Park et al., 1994]. Dexamethasone did not elevate the BBM enzyme activity, and inhibited the normal mucosal growth and mucosal hyperplasia in resected rats [Park et al., 1994]. Insulin-like growth factors and insulin itself are considered to be important regulators of somatic growth [Daughaday and Rotwein, 1989]. IGF-I and IGF-II have been reported to stimulate the proliferation of intestinal epithelial cells as well as to enhance the mucosal hyperplasia that occurs after massive small bowel resection [Park et al., 1994; Vanderhoof et al., 1992; Wheeler and Challacombe, 1997]. The serum insulin-like growth factor (IGF) levels were diminished in both groups (dexamethasone-infused resected and sham-operated rats) as well as the serum level of IGF binding proteins (IGF BP), the IGF BP-2 and IGF BP-3. However, the IGF BP-1 was increased and the IGF BP-4 did not change. This suggests that the growth inhibiting effects of dexamethasone in small intestinal mucosa may be partially mediated by decreasing serum IGF levels, or by alterations in IGF activity associated with changes in serum levels of IGF BP. Other findings confirm this hypothesis: glucocorticosteroids decreased the number of mitoses in both the gastric and duodenal mucosa, and reduced the weight of the stomach, small intestine and colon in growing rats. This process is reversed by IGF administration which induces proliferative events, such as the mitogenesis of intestinal cells [Steeb et al., 1995]. Dexamethasone enhanced the activity of disaccharidases in jejunum and ileum of sham-operated rats, and enhanced the sucrase and lactase activity in the duodenojejunum but not in the ileum of resected rats. Thus, glucocorticosteroids may control the site along the gastrointestinal tract responsible for nutrient digestion and absorption during intestinal adaptation via differential responsiveness of the proximal as compared with the distal bowel. The mechanisms involved in this process are unknown [Park et al., 1994].

Dexamethasone (0.2 mg/ g / day, given by infusion into the fetal amniotic cavity for seven days in a rabbit fetal gastroschisis model) increases the fetal small intestinal lactase activity by 70% [Guo et al., 1995]. However, the level of this enzyme is lower than in normal fetuses. The rate of glucose uptake is increased by 100% by dexamethasone, but again it is still lower than in normal fetuses.

The administration of dexamethasone (0.8 mg/150 g/ day SC for 2 days) was associated to a significant increase in bacterial adherence to the mucosa, accompanied by alterations in intestinal permeability. Thus, the bacterial-mucosal cell interactions may be responsible for the changes in the intestinal permeability after dexamethasone administration [Spitz et al., 1994].

The use of intestinal epithelial cell lines has allowed scientists to identify and characterize the importance of glucocorticosteroids on the induction and modulation of different genes involved in the physiological function of absorptive villous cells. Arrest of growth, formation of tight junctions, appearance of long microvilli, reorganization of endoplasmic reticulum and trans-Golgi network, and regulation of cell cycle by acting on cylin-dependent kinase 6 and p27^{Kip1}, are some of the characterized actions of glucocorticosteroids on enterocytes [Quaroni et al., 1999]. Although effects on differentiation were not observed in this *in vitro* model, we do not discard the possibility of glucocorticosteroids influencing development. Illustrations of this conclusion are presented in the next section.

3.22.b) Glucocorticosteroids and Intestinal Ontogeny

The human fetal serum hydrocortisone concentration doubles during the last 4 weeks of gestation [Murphy, 1982]. The release of corticosterone, a hormone similar to cortisol but less potent, has been observed to be one of the main factors in rat small intestinal maturation [Marti and Fernandez-Otero, 1994; McDonald and Henning, 1992]. This may represent a possible specific modulatory influence of glucocorticosteroid hormones on human fetal intestine. Several lines of evidence suggest this possibility: in immature animals, glucocorticosteroids induce precocious development of adult BBM activities, and facilitate the induction of specific enzymes by dietary carbohydrate. [Lebenthal et al., 1972; Nsi-Emvo et al., 1996; Sangild et al., 1995; Sheard and Walker, 1988]. The BBM activities of sucrase and isomaltase are not detectable in rats before 15 or 16 postnatal days. Glucocorticosteroids precociously induce the activity of α-glucosidases in the BBM. Sucrase and isomaltase are not detectable, nor are they induced by sucrose administration in adrenalectomyzed rats. And yet, the administration of glucocorticosteroids caused the appearance of these hydrolytic enzymes. After adrenalectomy, a decrease in the expected developmental rise of sucrase activity on the 17th postnatal day is observed, but on days 18, 21 and 28 no effects are seen [Henning and Sims, 1979]. After the administration of hydrocortisone (50 mg/g) on the 15th or 16th postnatal day, an increase in sucrase activity is observed. However, the hydrocortisone given on the 17th, 18th or 28th postnatal days does not affect sucrase activity. Neither adrenalectomy nor the administration of glucocorticosteroid have an effect on the activities of disaccharidases in the adult animal [Deren et al., 1967]. The rise in the sucrase-isomaltase mRNA after glucocorticosteroid administration indicates that these glucocorticoid effects on intestinal development are mediated by the crypt epithelial cells, and are due to the synthesis of new proteins, rather than to post-transcriptional enzyme activation [Nanthakumar and Henning, 1993]. The dosing of corticosteroid together with sucrose has a synergistic effect. Synergistical activity between corticosteroids and thyroxine on the induction of sucrase-isomaltase also has been observed [Leeper et al., 1998]. The adult values of BBM sucrase and isomaltase occur by the 30th postnatal day. At this time, the intestine is altered, so that it displays a more mature histological appearance [Lebenthal et al., 1972]. In preweaned intestine there is a parallel increase of corticosterone levels with that of the activity of ornithine decarboxylase activity (ODC) and ODC mRNA. Following these increases, the mucosal polyamine content also rises. It is speculated that polyamines mediate the premature expression of sucrase-isomaltase mRNA and sucrase activity [Nsi-Emvo et al., 1996; Rosewicz and Longsdon, 1991].

Different enzymes and functions mature after glucocorticosteroid administration, such as salivary amylase, pepsinogen, gastrin receptors, pancreatic amylase, ileal lysosomal hydrolases, jejunal and ileal pinocytosis, ileal bile salt transport, intestinal absorption of immunoglobulins, intestinal pyruvate kinase, hepatic tryptophan oxygenase, hepatic ornithine aminotransferase, glutamine synthetase and hepatic production of α-protein [Henning, 1981; Sarantos et al., 1994]. Thus, these effects show the glucocorticosteroid capacity of maturation in different functions and systems.

How is the sucrase-isomaltase (SI) in rat small intestine modulated by exogenous and endogenous glucocorticosteroids during 2nd and 3rd weeks? Although sucrase and isomaltase are distinct enzymes, they are synthesised as a single polypeptide from a 6-kb sucrase-isomaltase mRNA. Adrenalectomyzed rats on the 9th postnatal day had retarded appearance of sucrase, and the SI mRNA paralleled the enzyme activity. Administration of dexamethasone daily starting on the 10th postnatal day had a precocious appearance of SI and SI mRNA. Starting dexamethasone on the 16th day produces an accelerated rise in SI and SI mRNA; starting on 18th day did not have an effect. These results suggest that there are three distinct phases of glucocorticoid action: 1) an early phase, where activation of the gene occurs (probably around 10th postnatal day); 2) a late phase, where changes in cell kinetics occurs (probably around 16th postnatal day); and 3) a final phase, where there is a loss of responsiveness. It is likely that glucocorticosteroids regulate sucrase activity at a pretranslational level such as transcription and/or mRNA stability [Nanthakumar and Henning, 1993]. Similar results have been observed when analysing the trehalase mRNA expression after dexamethasone administration in a mouse model [Nitikul et al., 2001]. Trehalase mRNA is a marker of intestinal maturation in the mouse, being undetectable during the prenatal period, expressed at low levels during the first 2 weeks of postnatal life, and expressed at higher levels in the 3rd postnatal week [Nitikul et al., 2001].

The appearance and elevation of sucrase activity are preceded by a marked increase in its mRNA. However, even the mRNA increase is relatively slow (12-24 hours) after glucocorticoid administration. Glucocorticoid induction of sucrase in the epithelium is dependent on the underlying mesenchyme, and suggests that glucocorticosteroid most likely acts indirectly through one or more regulatory genes [Nanthakumar and Henning, 1993]. Cytokines such as transforming factor β1 (TGF-β1), β2 (TGF-β2), IL-1α and tumour necrosis factor α (TNF-α) are also developmentally regulated and may also be involved in the intestinal ontogeny [Schaeffer et al., 2000].

The thymidine analogue 5-bromo-2-deoxyuridine (BrdU) selectively inhibits differentiation in a number of tissues in suckling and mature animals. BrdU had no effect on BBM lactase and sucrase activity; thus, BrdU does not inhibit crypt-villus differentiation [Nanthakumar and Henning, 1995]. The administration of dexamethasone plus BrdU on the 10th postnatal day inhibited glucocorticoid-induced elevation of sucrase, trehalase and glucoamylase, but had no effect on lactase activity. This suggests that such maturation involves a different molecular mediator than does normal ontogeny. In contrast, administration of BrdU during normal development accelerated the ontogenic rise of sucrase and trehalase, as well as the ontogenic decline of lactase. This acceleration was also seen in adrenalectomized animals, which suggests that a glucocorticosteroid-independent component of normal intestinal ontogeny is activated by BrdU [Nanthakumar and Henning, 1995].

3.22.c) Effects of Glucocorticosteroids on Ion Transporters

In glucocorticoid deficient rats, Na^+ is not absorbed and Cl^- and HCO_3^- are secreted. In the group treated with methylprednisolone (40 mg per day for two days), more Na^+ was absorbed and more HCO_3^- was secreted. Also, the maximal secretory stimulus by 8-Br-cAMP of Cl^- secretion was similar in all three groups [Sellin and Field, 1981]. This suggests that glucocorticoids enhance Na absorption and HCO_3^- secretion, and do not affect the maximal secretory capacity of Cl^-. Corticosteroids are used in diarrheal diseases because of this proabsorptive effect, as well as for the antiinflammatory effect of this drug. The Na^+ absorption involves a primary increase in Na^+ entry across intestinal BBM of the intestinal epithelial cell, and also involves direct stimulation of sodium-potassium-adenosine triphosphatase activity (Na^+/K^+ ATPase) in the BLM. The mechanism by which glucocorticosteroids stimulate active intestinal Na^+ absorption is unclear, but in renal BBM glucorticosteroids stimulate the sodium/hydrogen (Na^+/H^+) exchange process [Donowitz et al., 1986]. Glucocorticoids increase the expression of the Na^+/H^+ exchanger in the ileum and proximal colon, but not in jejunum, distal colon or kidney [Cho et al., 1994; Yun et al., 1993]. Thus, in general the glucocorticosteroids stimulate the fluid absorption in duodenum, jejunum and ileum. There is no stimulation by glucocorticoids in the colon. Usually, there is an increase in net Na^+ absorption in proportion to the increase in the fluid absorption.

Phosphate is absorbed in the duodenum and jejunum, but phosphate absorption in the ileum and colon is negligible. The administration of cortisone increases phosphate absorption in the small intestine but not in the colon. Thus, the glucocorticoids enhance phosphate absorption in rat jejunum, where passive transport predominates. This suggests that glucocorticoids increase phosphate uptake not by enhancement of fluid movement, but by a direct effect on mucosal membrane permeability [Yeh and Aloia, 1987]. In contrast, a different study reported a decrease in phosphate transport in rats and chicks after glucocorticosteroid administration [Ferraro et al., 1976]. The concentration of phosphate used in this ligated loop study was 2 mM, which could be considered as a condition where active transport of phosphate predominates. This discrepancy might be explained by the hypothesis that glucocorticosteroids would have different effects on phosphate absorption in rat intestine, depending on the concentration of phosphate in the lumen, and therefore whether active or passive transport predominates [Yeh and Aloia, 1987].

The general glucocorticoid effect on calcium metabolism is a decrease in its intestinal absorption. However, corticoids increase the passive transport of Ca^{+2} in the proximal regions of the small intestine, where they compensate for their inhibitory effect on the active transport of Ca^{+2}. The clear dissociation between Ca^{+2} and phosphate absorption efficiency in the ileum suggests that the absorptive processes for Ca^{+2} and phosphate are separate in this region [Yeh and Aloia, 1987].

3.23) Clinical Aspects of the New Non Systemic Steroids

Corticosteroids are widely used to treat a variety of gastrointestinal and hepatic conditions such as ulcerative colitis, Crohn's disease and chronic active autoimmune hepatitis [Brattsand, 1990; Truelove, 1956; Truelove, 1957; Truelove, 1958; Matts, 1961; Mac Dougal, 1963; Danielson and Pritz, 1994; Tarpila et al., 1994; Nyman-Pantelidis et al., 1994; Lofberg et al., 1993; Hanauer et al., 1995; Lundin et al., 2001]. However, the systemically active steroids are associated with numerous potentially serious adverse effects [Haynes and Murad, 1985; Haynes and Larner, 1975; Baxter, 1979; Girdwood and Petrie, 1987; Enrich et al., 1992; Loeb et al., 1973; Delemos et al., 1970; Motoyama et al., 1971; Ballard et al., 1974; Sambhi et al., 1965, Krakoff et al., 1975; Shimidt et al., 1972; David et al., 1970, Nicollof, 1969; Okabe et al., 1971]. Even topical treatment with systemic steroids using rectal retention enemas or foams depresses plasma cortisol levels [Spencer and Kirsner, 1962; Farmer and Schumacher, 1970; Cann and Holdsworth, 1987; Reshef et al., 1992; Rodrigues et al., 1987].

The "perfect" steroid would have high topical glucocorticosteroid potency, low systemic bioavailability, rapid first pass metabolism in the intestine or liver, and rapid excretion. In this way, the numerous and potentially serious adverse effects might be minimized or avoided. To improve the poor topical anti-inflammatory action of systemic steroids such as betamethasone, dexamethasone and triamcinolone, lipophilic groups are introduced in the 16-α and/or 17-α positions of the steroid nucleus. Betamethasone 17-valerate and triamcinolone acetonide have been synthesized and used to treat patients with certain dermatological diseases as well as asthma (Mygind and Clark, 1980). Having lipophilic groups, these corticosteroids are easily inactivated in the liver, which results in less circulating corticosteroids and consequently fewer systemic adverse effects [Mygind and Clark, 1980; Martin et al., 1975; Jewell and Phill, 1990]. Glucocorticosteroids with 16α, 17α acetyl groups, like budesonide and fluticasone propionate [Brattsand et al., 1982; Clissold and Hell, 1984; Bauer et al., 1988], have features which facilitate their topical anti-inflammatory activity and stability in extra-hepatic tissues such as the airways and intestinal walls. This confers about 90% first-pass hepatic metabolism of the parent drug to metabolites which posses minimal or no biological activity [Brattsand, 1990; Greenberg, 1994].

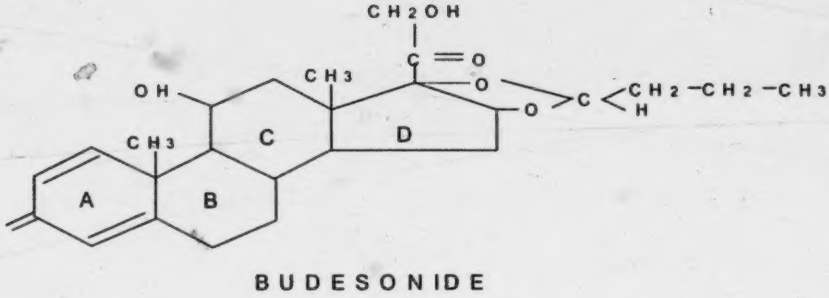
Tixocortol pivalate, a synthetic corticosteroid, has high first-pass metabolism, but does not have high topical corticosteroid potency [Juniem, 1988]. Fast and extensive transformation of tixocortol pivalate into inactive metabolites provides an explanation for the large dissociation between the topical and systemic activities of this drug [Chanoine et al., 1987; Chanoine and Juniem, 1984]. The reduced intestinal absorption of tixocortol pivalate may be an important factor to explain its low topical activity [Chanoine, 1988]. Tixocortol is an effective steroid for topical use in patients with ulcerative colitis, and is not associated with adverse reactions [Hanauer, 1988; Larochelle, 1983]. Tixocortol has also been proposed to have a better side-effect profile as compared to systemic steroids when given orally in the treatment of Crohn's disease [Kocian and Kocianova, 1999].

Beclomethasone dipropionate, fluticasone propionate and budesonide are called 17-α substituted glucocorticosteroids. These drugs have high topical activity (much greater than that of tixocortol pivalate), and have rapid first-pass metabolism in the liver. Fluticasone propionate has a systemic bioavailability which is extremely low as a result of extensive hepatic first-pass metabolism. Fluticasone has a topical potency two-times greater than that of beclomethasone dipropionate. Its oral bioavailability is low due to its low absorption from the intestine [Brattsand, 1990]. In an uncontrolled trial, the administration of an oral daily dose of 20 mg fluticasone propionate was shown to be effective to treat patients with mild to moderately active small intestinal and colonic Crohn's disease [Carpani de Kaski et al., 1991]. In contrast, two controlled studies failed to show any benefit of fluticasone in the treatment of patients with ulcerative colitis [Hawthorne et al., 1993, Wright et al., 1993].

Beclomethasone dipropionate has a topical activity 100-times greater than that of tixocortol pivalate. Beclomethasone dipropionate is metabolized in the liver, resulting in oxidative products and beclomethasone which retain corticosteroid activity [Axelsson B. et al., 1984]. Beclomethasone is effective in the treatment of patients with proctosigmoiditis [Lofberg et al., 1993, Van der Heide et al., 1988; Van der Heide et al., 1987; Levine and Rubin , 1985; Halpern et al., 1991].

Budesonide, a non-systemic glucocorticosteroid with substitution at the 17α ester position, has high topical activity, double that of beclomethasone dipropionate. It is 100-times more water soluble than beclomethasone and tixocortol, which facilitates its intestinal absorption [Brattsand, 1990; Greenberg, 1994; Edsbadker et al., 1987a; Edsbadker et al.,1987b]. Budesonide is metabolised in the liver, and has a 90 % first-pass metabolism which allows only about 10 % of intact budesonide to reach the systemic circulation. Down-regulation of the expression of IL-6 mRNA in rat intestinal muscle cells and in splenic monocytes by budesonide *in vitro* illustrates the anti-inflammatory effect of this non-systemic steroid [Deng et al., 1996]. In addition, the inhibition in *vitro* of the release of pro-inflammatory cytokines by intestinal lamina propria mononuclear cells from colonic biopsies as well as peripheral monocytes also demonstrates the budesonide's anti-inflammatory activity [Schreiber et al., 1996].

Figure 9. Budesonide



Physical Chemical Properties:

1. 90 % fist-pass metabolism
2. High topical activity
3. 100 times more water soluble than tixocortol and beclomethasone

3.24) Topical Budesonide

Budesonide has been used for the treatment of patients with distal ulcerative colitis, proctitis and Crohn's disease (Table 2). Good clinical results have been observed with budesonide enemas (2 mg/100 ml) [Danielsson et al., 1992; Matzen, 1991; Hanauer et al., 1995; Pruitt et al., 1996; Cortot et al., 2001a]. When given as an enema, budesonide does not cause alterations in the levels of endogenous plasma cortisol [Halpern et al., 1991; Pruitt R. et al., 1996; Palmen et la., 1998]. In distal colitis, budesonide enemas are equivalent in efficacy to 5-ASA enemas, or to enemas containing prednisone, methyl prednisolone or hydrocortisone [Mac Dougal, 1963, Danielsson et al., 1992, Lofberg et al., 1994; Leman et al., 1995; Bianchi Porro et al., 1994; Bayless et al., 1995]. Low viscosity budesonide enemas are more effective in patients with ulcerative colitis and proctitis than are the high viscosity enemas, presumably because the former spread over a larger area of the bowel [Nyman-Pantelidis et al., 1994]. It is suggested that a hydrophobic suppository with prednisolone should be used for patients in whom inflammation is confined to the rectum, and a hydrophilic suppository used for patients in whom inflammation reaches the rectum and the middle part of the sigmoid colon [Sadahiro, 1992].

In Canada, budesonide enemas are approximately the same cost as betamethasone or 5-ASA enemas. The comparative studies performed to date have not considered the total consumption of enemas over an extended time, such as 12 months. Because of the high recurrence rate of distal colitis/proctitis, and the need for some patients to use enemas continuously or intermittently, this becomes an important issue when considering cost and efficacy.

Table 2. Clinical Trials of Budesonide Eenemas in Distal Colitis

Budesonide enemas vs:	Author	Outcome
placebo	Danielsson 1992 Hanauer 1995	Bud > PL Bud > PL
prednisolone	Matzen 1991 Lofberg 1994	dose-ranging study Bud = Pred
methyl prednisolone	Bianchi Porro 1994	Bud = MPred
hydrocortisone	Tarpila 1994 Bayless 1995	Bud = Hc Bud = Hc
5-ASA	Lamers 1991 Leman 1995	Bud = 5-ASA Bud = 5-ASA

Abbreviations: Bud: budesonide; Hc: hydrocortisone; MPred: methyl prednisolone;

P: prednisolone; PL: placebo; 5-ASA: 5-aminosalicyclic acid

In a recent meta-analysis of the efficacy of budesonide enemas in distal ulcerative colitis, this drug has shown to be as effective as conventional rectal steroids, with less suppression of endogenous cortisol production [Marshall et al., 1996].

3.25) Oral Budesonide

The controlled ileal release (CIR) form of the budesonide taken by mouth is composed of a gelatine capsule involving multiple acid-stable microgranules which contain an inner sugar core surrounded by budesonide, and an outer coat of Eudragit L100-55 that dissolves in the small intestine at pH 5.5 or higher. Budesonide CIR is targeted for absorption in the ileo-cecal region. From 52% to 79% is absorbed, with a mean absorption time of 6.4 hours, and a systemic bioavailability of only 9% [Edsbacker et al., 1987]. Budesonide CIR has been used in patients with Crohn's disease, with good clinical results [Lofberg et al., 1993, Greenberg et al., 1994; Rutgeerts et al., 1994; Campieri et al., 1995; Levine et al., 2001; Tremaine et al., 2001; Cortot et al., 2001b;

Cortot et al., 2001c; Cortot et al., 2001d; Cortot et al., 2001e; Biancone and Pallone, 1999; Lang and Peppercorn, 1999; Rutgeerts, 2001]. For example, Greenberg and colleagues [Greenberg et al., 1994] tested the efficacy of varying doses of budesonide CIR in patients with active Crohn's disease: remission occurred in 51% of patients receiving 9 mg of budesonide, in 43% of those receiving 15 mg and in 33% of those receiving 3 mg of budesonide; the 9 and 15 mg doses were statistically superior to placebo. Although budesonide causes a slight alteration in basal and corticotropin-stimulated plasma cortisol concentrations, this drug was not associated with clinically important adverse effects [Greenberg et al., 1994; Nos et al., 2001]. Rutgeerts and co-workers (Rutgeerts et al., 1994; Campieri et al., 1995] assessed the efficacy of budesonide versus prednisolone in patients with active ileal or ileocecal Crohn's disease. The efficacy of prednisolone was greater than budesonide in terms of the patient's Crohn's disease activity index (CDAI): after 10 weeks of therapy, 53% of the patients receiving budesonide were in remission (determined as a CDAI score below or equal to 150), whereas 66% of patients receiving prednisolone were in remission. However, budesonide was associated with fewer side effects, as well as fewer effects on pituitary-adrenal function. Furthermore, two patients in the prednisolone group had serious complications of intestinal perforation and an abdominal-wall fistula [Rutgeerts et al., 1994]. In pediatric patients with Crohn's disease modest effectiveness and subnormal growth were observed with CIR budesonide preparations [Kundhal et al., 2001].

In a thoughtful editorial [Sachar, 1994], Sachar raised three interesting points: firstly, in patients with active Crohn's disease, the peak remission rates were not achieved with budesonide before eight weeks, whereas in the European study the remission rates with prednisolone were maximal by four weeks. Secondly, in the absence of a unified study including budesonide, prednisolone and placebo groups, the clinical importance of the lower incidence of systemic effects with budesonide is uncertain and difficult to assess; thirdly, if budesonide truly has fewer side effects than conventional corticosteroids, this may commensurate with a somewhat slower action and lower therapeutic efficacy .

Systemic effects have been reported with budesonide CIR 4.5 mg b.i.d. for 8 weeks and then with 4.5 mg once in the morning for 2 weeks. These systemic effects were assessed by cortisol excretion and plasma concentration of budesonide [Naber et al., 1996]. In relation to the suppression of osteoblastic function, budesonide CIR is superior to conventional steroids, being considered safer for long-term use than systemic steroids [D' Haens et al., 1996; Papi et al., 2000]. Systemic activity of CIR budesonide (200 µg/kg/day) in rats has also been demonstrated. However, this steroid was very effective in reducing intestinal inflammation [Boyd et al., 1995].

Budesonide may also be useful to delay the time to recurrence of attacks of Crohn's disease [Greenberg et al., 1996; Lofberg et al., 1994; Cortot et al., 2001b; Cortot et al., 2001c; Cortot et al., 2001d; Steinhart et al., 2000; Cortot et al., 2001 e]. However, by one year only one-third of the treatment and placebo groups were still free of relapses. This is important, since repeated relapses of Crohn's disease clearly increase morbidity of the disease [Bayless, 1996; Simms and Steinhart, 2001].

Oral budesonide (6 mg once daily) offers no benefit in preventing endoscopic recurrence after surgery for ileal/ileocecal fibrostenotic Crohn's disease, but it may be useful in patients who have undergone surgery for disease activity [Heller et al., 1996]. Thus, budesonide is an effective therapy for the treatment of patients with Crohn's disease, without the risk of as many adverse effects [Rutgeerts, 2001].

3.26) Budesonide in Hepatitis , Ulcerative Colitis and Intestinal Transplantation

In an uncontrolled trial, it was shown that oral budesonide induces a remission in patients with autoimmune chronic active hepatitis, with low frequency of corticosteroid-related adverse effects [Lofberg et al., 1993]. Controlled colonic release budesonide may be useful in patients with active ulcerative colitis [Lofberg et al., 1994]. A controlled colonic release form of prednisolone metasulphabenzoate (Eudragit-coated prednisolone) may be useful for the treatment of extensive ulcerative colitis, without the risk of systemic steroid side-effects [Mc Intyre et al., 1985, Ford et al., 1992].

The administration of pH-modified release capsules of budesonide (Budenofalk 3mg) targeting colonic therapy has little effect on blood lymphocytes and granulocytes, indicators for systemic side effects [Moellmann et al., 1996]. This preparation has been used with effectiveness in acute intestinal graft versus host disease (GVHD) [Betz et al., 1999].

An oral glucocorticoid-conjugate, budesonide-beta-D-glucuronide, which is not absorbed in the rat small intestine but is hydrolyzed by colonic bacterial and by mucosal beta-glucuronidase, releases free budesonide into the colon. This decreases the bioavailability of this drug, and consequently decreases its side effects [Mc Leod et al., 1994; Tozer et al., 1991; Cui et al., 1994]. More work is needed to determine whether this delivery system may be an alternative for treating patients with inflammatory bowel disease.

Budesonide has been demonstrated to be a useful immunosuppressive agent for clinical intestinal transplantation. This agent increases the immunosuppressive effect of cyclosporin A and prolongs small bowel allograft survival in rats without the expected systemic activity of glucocorticosteroids (inhibition of normal ACTH release) [Ozcay et al., 1997].

3.27) Conclusions

The non-systemic steroid budesonide is useful for the treatment of distal colitis when given by enema, and for Crohn's disease when given by mouth. Budesonide's adverse effect profile is superior to conventional glucocorticosteroids. Further applications may include the management of patients with ulcerative colitis or autoimmune chronic active hepatitis.

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D) HYPOTHESES

I reviewed in detail the potential links between glucocorticosteroids, dietary lipids and the process of adaptation of nutrient transport, including short bowel syndrome. Based on this background I propose the following hypotheses:

- Systemically and locally acting glucocorticosteroids enhance the intestinal absorption of sugars and lipids.
- Feeding a saturated fatty acid diet will enhance the effect of steroids on the intestinal uptake of nutrients.
- Systemically and locally acting glucocorticosteroids enhance the intestinal absorption of sugars and lipids after intestinal resection.
- Feeding a saturated fatty acid diet will enhance further the intestinal absorption of sugars and lipids after intestinal resection.
- The alterations in sugar absorption may be explained by the expression of specific transporters related to these specific nutrients
- The alterations in lipid absorption may be explained by the active component of lipid uptake, or in other words by the expression of fatty acid binding proteins.
- The possible signals responsible for this adaptive response are enteroglucagon, ornithine decarboxylase (ODC), early response genes (ERG) such as c-myc, c-jun and c-fos, and cytokines such as TNF-α, IL-2, IL-6 and IL-10.

The rat animal model was used in the experiments based on the literature review where most of the studies describe the uptake and effect of steroids on rat small intestine. Weanling animals were chosen because this specific aging period involves dramatic changes in terms of intestinal uptake. Exactly at the third week, the animals need to obtain all their nutrients from a different source other than the mother's milk. This period of transition has been highly associated to the high peak of physiological corticosterone found at the period time. We speculate that administrating steroids to weanling animals until they reach adult life, that we would find more effects than administrating steroids in adult life in function of their higher sensitivity to steroids. This sensitivity to steroids has been correlated with the amount of glucocorticoid receptor present in the cell's cytoplasm.

Figure 10. Summary of Hypotheses

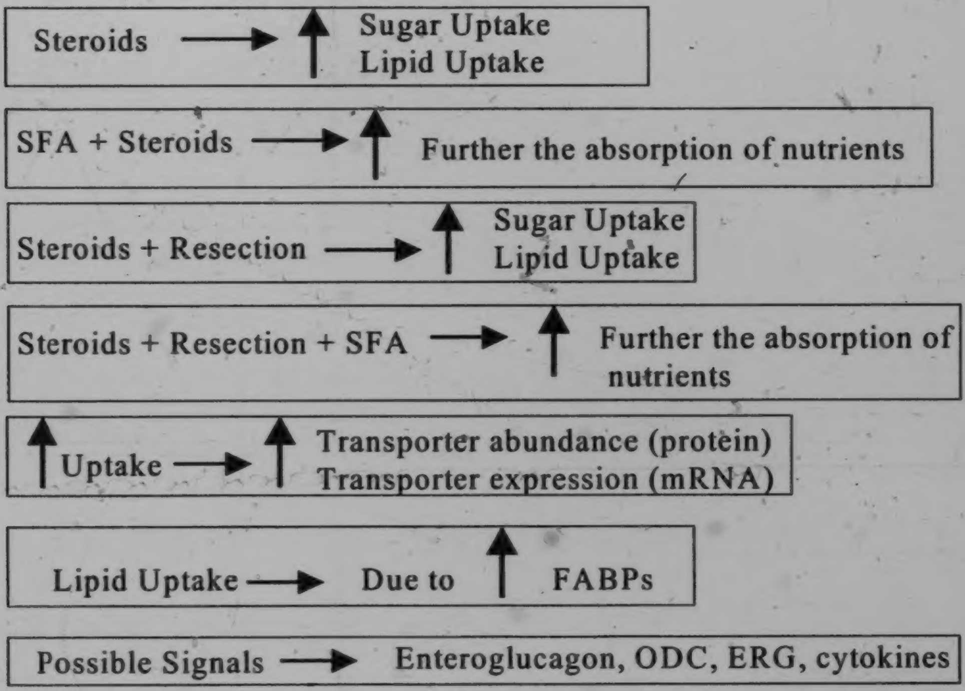
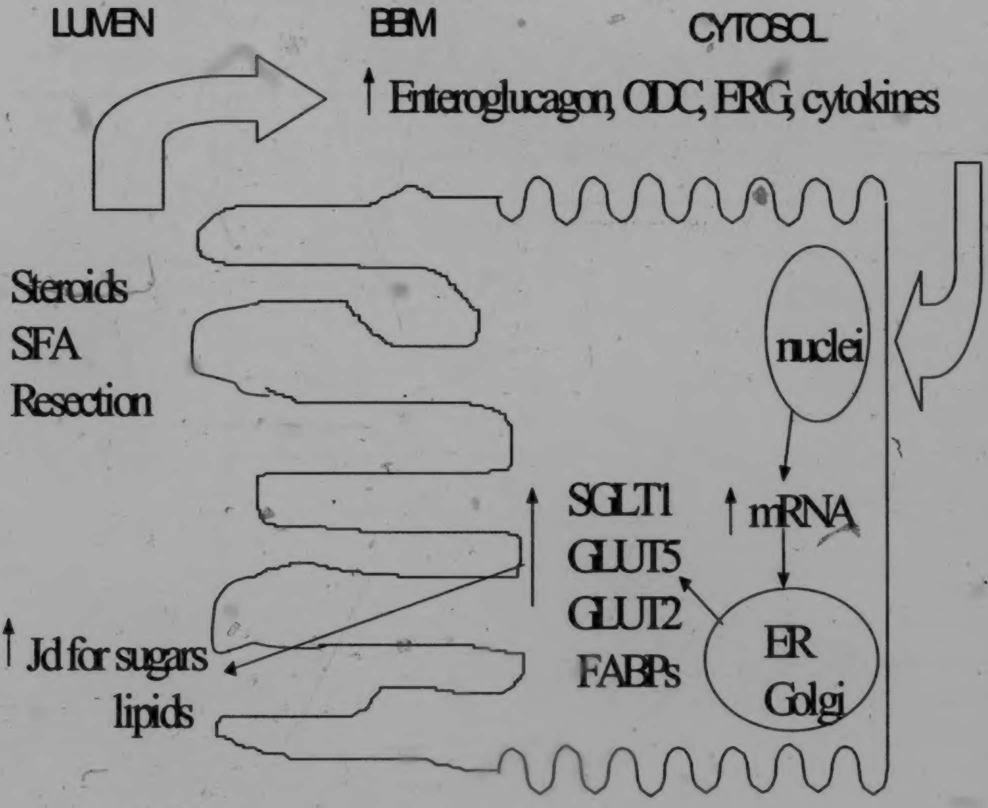

Steroids → ↑ Sugar Uptake
Lipid Uptake
SFA + Steroids → ↑ Further the absorption of nutrients
Steroids + Resection → ↑ Sugar Uptake
Lipid Uptake
Steroids + Resection + SFA → ↑ Further the absorption of nutrients
↑ Uptake → ↑ Transporter abundance (protein)
Transporter expression (mRNA)
Lipid Uptake → Due to ↑ FABPs
Possible Signals → Enteroglucagon, ODC, ERG, cytokines

Figure 11. Summary of Hypotheses


LUMEN
BBM
CYTOSOL
↑ Enteroglucagon, ODC, ERG, cytokines
Steroids
SFA
Resection
nuclei
↑ mRNA
↑ SGLT1
GLUT5
GLUT2
FABPs
ER
Golgi
↑ Jd for sugars
lipids

E) METHODS

1) Animals and Diets

The principles for the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies and by the Council of the American Physiological Society, were carefully observed in the conduct of this study. Weanling male Sprague Dawley rats, 21-23 days of age *post partum*, were obtained from the University of Alberta Vivarium. Pairs of rats were housed at a temperature of 21°C, with 12 hours of light and 12 hours of darkness. Water and food were supplied *ad libitum*. The animals were fed one of three diets: a fed standard Purina^R rat chow, a semisynthetic diet with 20% (w/w) fat enriched in either polyunsaturated (PUFA) or saturated (SFA) fatty acids corresponding to a high or low polyunsaturated-to-saturated fatty acid ratio, respectively. The weaning rats were fed chow, SFA or PUFA for 4 weeks. These isocaloric semi-synthetic diets were nutritionally adequate, providing all known essential nutritional requirements. PUFA provided approximately 22 % of calories and 69 % of total fatty acids (% w/w) as 18:2w6 (linoleic acid), whereas SFA provided 22 % of calories and 9.6 % of total fatty acids as 18:2w6.

The animals were sacrificed by the injection of sodium pentobarbitol (240 mg/100 g body weight). The whole length of the small intestine was removed rapidly. The proximal third was termed the jejunum and the distal third the ileum; the middle third was discarded.

Table 3. Macronutrient Composition of the Semisynthetic Diets

INGREDIENTS	CONCENTRATION (g/kg DIET)
Fat	200.00
Cornstarch	378.00
Casein	270.50
Non-nutritive fiber	80.00
Vitamin mix*	10.00
Mineral mix#	50.00
L-methionine	2.50
Choline	2.75
Inositol	6.25

*A.O.A.C. vitamin mix (Teklad Test Diets, Madison, WI) provided the following per kilogram of complete diet: 20,000 IU of vitamin A; 2,000 IU of vitamin D; 100 mg of vitamin E; 5 mg of menadione; 5 mg of thiamine-HCl; 8 mg of riboflavin; 40 mg of pyridoxine-HCl; 40 mg of niacin; 40 mg of pantothenic acid; 2,000 mg of choline; 100 mg of myoinositol; 100 mg of p-aminobenzoic acid; 0.4 mg of biotin; 2 mg of folic acid; and 30 mg of vitamin B12.

#Bernhart Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH) was modified to provide 77.5 mg of Mn and 0.06 mg Se per kilogram of complete diet.

Table 4. Fatty Acid Composition of The Semisynthetic Diets

FATTY ACID (% of total)	SFA	PUFA
14:0	1.8	0.1
14:1ω9	0.0	0.0
15:0	0.2	0.0
16:0	21.8	5.5
16:1ω7	0.0	0.1
18:0	61.1	5.2
18:1ω9	2.8	16.3
18:1ω7	0.1	0.2
18:2ω6	9.6	69.2
18:3ω3	0.0	0.8
18:3ω6	0.0	0.1
18:4ω3	0.0	0.0
19:0	0.2	0.0
20:0	1.5	0.2
20:1ω9	0.0	0.3
20:1ω7	0.1	0.3
20:2ω6	0.1	0.2
20:3ω9	0.1	0.2
20:3ω6	0.0	0.0
20:4ω6	0.0	0.0
20:5ω3	0.0	0.3
22:0	0.0	0.4
22:1ω9	0.0	0.0
22:1ω7	0.3	0.3
22:4ω6	0.0	0.1
22:5ω6	0.0	0.0
22:5ω3	0.1	0.1
24:0	0.1	0.1
22:6ω3	0.1	0.0

2) Drugs

There were 24 animals in each of three drug groups: control (CON, 0.19% EDTA buffered saline), budesonide (BUD, 0.25 mg/kg body weight per day), and prednisone group (PRED, 0.75 mg/kg body weight per day). The doses of PRED and BUD were chosen on the basis of regimens which have been shown to be useful clinically [Bratssand, 1990; Rutgeerts et al., 1994; Greenberg et al., 1994]. These doses are similar to the doses used to treat trinitrobenzene sulphonic acid ileitis in rats [Boyd et al., 1995] and are lower than the doses used to prevent graft rejection in a rat model of intestinal transplantation (1.0 mg/kg/day) [Ozcay et al., 1997]. Within each drug group there were eight rats fed Chow, eight fed PUFA, and eight fed SFA. The drugs were administrated each day by oral gavage, and were dissolved in 0.19% EDTA buffered saline. The volume of vehicle given was 5 µl/g body weight. The oral dosing was performed at 12:00 h daily including weekends, and was continued for 4 weeks.

The resected animals were divided into four groups, with 6 animals in each diet group (Chow, PUFA and SFA): control vehicle (0.19% EDTA buffered saline), budesonide (0.25 mg/kg body weight per day), prednisone (0.75 mg/kg body weight per day), and dexamethasone (128 mg/kg body weight per day). The control vehicle, budesonide and prednisone were administrated by oral gavage and were dissolved in 0.19% EDTA buffered saline, whereas dexamethasone was administrated subcutaneously and was dissolved in bacteriostatic 0.9% sodium chloride solution.

3) Morphology

To prepare the histological sections, the samples of the jejunum and ileum were dehydrated, embedded in paraffin, sectioned for light microscopy, and stained with hematoxylin and eosin using standard techniques. Multiple histological cross-sections were scanned for areas in which the section passed through the entire vertical length of villus and crypts. The areas of longitudinal cross-section of villi were measured using the Magiscan-GENIAS image analysis program (JOYCE-LOBEL, 1987 England) in at least 10 well-oriented crypt-villous systems for each animal group.

4) Probes and Marker Compounds

The [^{14}C]-labelled probes included lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18;1), linoleic acid (18:2), linolenic acid (18:3) (each at 0.1 mM), cholesterol (0.05 mM), L-glucose (16 mM), D-mannitol (16 mM), and varying concentrations of D-glucose and D-fructose (4, 8, 16, 32 and 64 mM). The long-chain fatty acids and cholesterol were solubilized in 10 mM taurodeoxycholic acid [Thomson and O'Brien, 1981]. Unlabelled and [^{14}C]-labelled probes were supplied by Sigma Co. (St Louis, Missouri) and by New England Nuclear, respectively. [^{3}H]-inulin was used as a non-absorbable marker to correct for the adherent mucosal fluid volume. Probes were shown by the manufacturer to be more than 99% pure by high performance liquid chromatography.

5) Tissue Preparation and Determination of Rates of Uptake

The animals were sacrificed by the injection of sodium pentobarbitol (240 mg/100 g body weight). The whole length of the small intestine was removed rapidly. The proximal third was termed the jejunum and the distal third the ileum; the middle third was discarded. The intestine was everted and cut into small rings of length of approximately 2-4 mm each. The rings were immersed immediately in preincubation beakers containing oxygenated Krebs-bicarbonate buffer (pH 7.2) at 37^0C, and were allowed to equilibrate prior to commencement of the uptake studies. Uptake was initiated by the timed transfer of tissue rings to a shaking water bath (37^0C) containing 5 ml plastic vials with gassed Krebs buffer, plus [^{3}H]-inulin and the [^{14}C]-labelled substrates. After incubation for 5 min, the uptake of nutrient was terminated by pouring the vial contents onto filters immobilized on an Amicon vacuum filtration manifold maintained under suction. This was followed by washing jejunal or ileal rings with ice-cold saline. The tissue was dried and the weight was recorded prior to saponification with 0.75 N NaOH. Scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes.

6) Expression of the Results and Statistics

The rates of uptake were expressed as nmol of substrate taken up per 100 mg dry weight of tissue per minute (nmol*100 mg tissue^{-1}*min^{-1}). The values obtained from the two dietary groups (Chow, SFA or PUFA), and from the three or four treatment groups (control [CON], prednisone [PRED] or budesonide [BUD] or dexamethasone [DEX]), are reported as the mean ± SEM of results obtained from eight or six animals in each group depending on the experiment.

The values of the maximal transport rate (Vmax) and the apparent Michaelis constant (Km) were estimated using non-linear regression software (Sigma Plot program, Jandel Scientific, San Rafael, CA). Because there was a linear relationship between fructose concentration and uptake, the values of Vmax and Km could not be calculated.

For this reason, linear regression was used to obtain the value of the slope of this linear relationship. The ANOVA one way and Student Newman-Keul test were used to determine the significance of the differences among the means of animals fed Chow and treated with CON, BUD, PRED or DEX. The ANOVA two ways and Student Newman-Keul test were used to test the animals fed SFA and PUFA, and given CON, BUD or PRED, as well as the animals submitted to intestinal resection, fed SFA and PUFA, and given CON, BUD, PRED or DEX. A p value of 0.05 or less was accepted as representing a statistically significant difference.

7) Surgical model

The rats were exposed to halothane (5.0%) until limp, when the hair on the anterior abdominal wall was clipped and the skin was cleansed with betadine. The animals were kept sedated under halothane (0.5-1.5%) throughout surgery. The sleeping animal was restrained in the supine position on an animal operating board, using rubber band leg loops. A heating pad was maintained at 37°C under the operating board, with circulating water from a water bath during all subsequent surgical procedures. Following a ventral incision along the linea alba, the middle 50% of the small intestine was removed from half of the animals. The other half of the rats had a transection, i.e., the small intestine was divided and then reanastomosed, without removal of any portion of the intestine. Sterile instruments and an aseptic technique were used. The colon was located, and the distance from the ileo-cecal valve to the ligament of Treitz was measured with 5-0 silk string. The string was cut in half to give the approximate measure of the length of intestine to be resected. The intestinal portion to be resected was determined by placing one end of the measuring string at 2 cm after ileal-cecal valve (to prevent back flow of bacteria from colon), and working the string along the intestine towards the jejunal end. In the resected animals, 50% of the middle portion of the intestine was removed, leaving the proximal 25% and the distal 25%. Bowel continuity was restored by an end-to-end jejunoileal anastomosis using interrupted 6-0 silk sutures. The abdomen was closed with a continuous 3.0 dexon suture. After surgery, the animals received a subcutaneous injection of buprenorphine, 0.01-0.05 mg/kg body weight, for pain relief. The animals recovered in clean plastic cages under a heat lamp, and were then taken to fresh cages where they were housed individually.

8) References

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F) LOCALLY AND SYSTEMICALLY ACTIVE GLUCOCORTICOSTEROIDS MODIFY INTESTINAL ABSORPTION OF SUGARS IN RATS

1) Introduction

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis [Lofber et al., 1993; Nyman-Pantelides et al., 1994; Tarpila et al., 1994; Girdwood and Petrie, 1987; Murphy, 1982; Batt and Peters, 1976]. However, the systemically active steroids may be associated with potentially serious adverse effects [Lofberg et al., 1993; Cann and Holssworth, 1987; Reshef et al., 1992; Rutgeerts et al., 1994]. The high prevalence of these adverse effects has been a major impetus for the development of non-systemic steroids. Budesonide is a non-systemic steroid with high topical activity, low systemic bioavailability, and rapid first pass metabolism in the intestine and liver [Brattsand, 1990; Thiesen and Thomson, 1996]. Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease [Brignola et al., 1994; Greenberg et al., 1994; Danielson et al., 1992].

In young animals steroids induce precocious development of some of the intestinal brush border membrane (BBM) enzymes, and facilitate the induction of specific enzymes by dietary carbohydrate [Haynes and Murad, 1985; Lebenthal et al., 1972; Batt and Peters, 1976b]. Systemically active steroids given by mouth enhance glucose uptake by adult animals [Batt and Scott, 1982]. Dexamethasone (128 mg/kg/day) given subcutaneously for seven days blunts the expected adaptive response following intestinal resection [Park et al., 1994].

The Na^+ -gradient across the BBM provides the driving force for glucose transport [Greenberg, 1994; Ricklis and Quastel, 1958; Wright, 1992]. This gradient is maintained by the action of the Na^+/K^+-ATPase, which is restricted to the basolateral membrane of the enterocyte [Hirayama et al., 1992]. SGLT1 mediates the BBM Na^+/glucose cotransport [Vehyl et al., 1992; Vehyl et al., 1993; Weber et al., 1991]. Fructose uptake across the BBM is mediated by facilitated diffusion by GLUT5 [Burant and Bell, 1992; Burant et al., 1992; Rand et al., 1993; Shu et al., 1997], whereas GLUT2 mediates the facilitative Na^+-independent diffusion of glucose and fructose through the BLM. [Caspary and Crane, 1968] Recent evidence suggests that GLUT2 may also be in the BBM [Kellet and Helliwell, 2000; Helliwell et al., 2000; Helliwell et al., 2000b].

Proglucagon-derived peptides originate from processing and breakage of the proglucagon gene product [Mojsov et al., 2000; Orskov et al., 1987] in the L-cells present in the ileum and colon [Larsson et al., 1975]. Ornithine decarboxylase (ODC) is a key enzyme in the synthesis of polyamines, a requirement for any proliferative event. Early response genes (ERG) are genes expressed in response to proliferative stimulation. It has been suggested that the mRNA levels of proglucagon and ornithine decarboxylase as well as the mRNAs of early response genes such as c-myc, c-jun and c-fos may be involved in the intestinal adaptive process such as resection of the small intestine [Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998]. It is unknown if proglucagon, ODC or ERGs in the intestine are influenced by steroids.

A wide variety of cytokines are produced locally by the intestinal epithelium, and they are involved in the homeostasis of the intestinal tissue during development [Fiochi, 1997; Kagnoff et al., 1996]. Cytokine gene expression has been shown to be regulated by hydrocortisone and dexamethasone during postnatal small intestinal development [Schaeffer et al., 2000]. Cytokines alter sugar absorption [Hardin et al., 2000; Berridge and Tan, 1995; Taylor et al., 1992]. It is not known if the cytokines in the intestine are influenced by prednisone or budesonide.

Accordingly, this study was undertaken to assess the influence of budesonide and prednisone, in doses equivalent to those used in clinical practice, on 1) the intestinal uptake of glucose and fructose in young growing rats; 2) the abundance of the glucose and fructose transporter proteins and the expression of their respective mRNAs; and 3) the mRNA expression of several potential signals of steroid-associated intestinal adaptation including proglucagon, ODC, three ERGs (c-myc, c-fos and c-jun), and selected cytokines (TNF-α, IL-2, IL-6 and IL-10).

2) Methods and Materials

2.1) Membrane preparation

There were 8 animals in each of the 3 drug groups (control, budesonide and prednisone). Two 40 cm lengths of proximal jejunum and distal ileum were rapidly removed and rinsed gently with ice-cold saline. The intestine was opened along its mesenteric border, and the mucosal surface was washed carefully with cold saline to remove mucus and debris. The mucosal surface was blotted with lint-free tissue to remove excess moisture, and was removed from the rest of the intestinal wall by gently scraping with a microscopic slide and then snap-freezing the tissue in liquid nitrogen at -80°C for later membrane preparation. Brush border membranes (BBM) and basolateral membranes (BLM) were isolated from the rat intestinal mucosal scrapings using homogenization, differential centrifugation, and Ca^{2+} precipitation [Maenz and

Cheeseman, 1986; Orsenigo et al., 1985; Orsenigo et al., 1987]. Aliquots were stored at -80°C for Western immunoblotting.

2.2) Western immunoblotting

BLM and BBM proteins were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). After electrophoresis, proteins were immobilized and transferred to nitrocellulose by electroblotting. Then, the membranes were blocked by incubation overnight in 5% w/v dry milk in Tween Tris Buffered Saline (TTBS: 0.5% Tween 20, 30mM Tris, 150 mM NaCl). Membranes were subsequently washed three times with TTBS and probed with specific rabbit anti-rat antibodies: α1-Na^+/K^+ ATPase, β1-Na^+/K^+ ATPase, GLUT2, GLUT5 and SGLT1. The antibodies were diluted in 2% dry milk in TTBS and the incubations were done at room temperature.

The polyclonal antibodies against SGLT1 and GLUT2 were obtained from Biogenesis, Poole, England. The polyclonal antibody against GLUT5 was obtained from Chemicon International Inc., Temecula, California. The polyclonal antibodies anti-rat α1 and β1-Na^+/K^+ ATPase were obtained from Upstate Biotechnology Inc., Lake Placid, NY.

Following incubation in primary antibody, membranes were washed three times with TTBS. Membranes were then incubated with goat anti-rabbit antibody conjugated with horseradish peroxidase, HRP, (Pierce, Rockfort, Illinois, USA). After three washes in TTBS, the immune complexes were visualized with SuperSignal® Chemiluminescent-HRP Substrate (Pierce, Rockfort, Illinois, USA). After exposure to X-OMAT AR film, the relative band densities were determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life science group, Cleveland, Ohio, USA).

2.3) Northern Immunoblotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E. coli*) were transformed with plasmids containing the desired DNA sequences to be probed for the Northern blotting. SGLT1 cDNA probe was donated by Dr. Davidson, University of Chicago; cDNA probes encoding the α1 and β1 Na^+/K^+ ATPase subunit isoforms were obtained from Dr. Lingrel, University of Cincinnati; cDNA probes encoding GLUT5 and GLUT2 were obtained from Dr. Bell, University of Chicago; ERG probes were obtained from Oncogene Research Products; cDNA probe encoding proglucagon was obtained form Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; ODC was obtained from Dr. Blackshear, University of Chicago; and TNF-α, IL-2, IL-6 and IL-10 were obtained from BIO/CAN Scientific. A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labelled DNA (Roche Diagnostics, Quebec, CA).

RNA was extracted from the mucosal scrapings of the jejunum and ileum obtained from at least three animals in the 3 groups. These intestinal segments were homogenized in a denaturing solution containing guanidinium thiocyanate, using a biorad fast prep shaking centrifuge. Following addition of 2M sodium acetate, a phenol chloroform extraction was performed. The upper aqueous phase was transferred to a tube, and the RNA was precipitated with isopropanol and washed with 70% ethanol. RNA samples were stored at -70°C.

Total RNA was electrophoresed through a denaturing agarose gel (1.16%

agarose) and then transferred from the gel to a nylon membrane by capillary action, overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA onto the membrane. As a pre-hybridization, membranes were incubated for 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA). Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at the adequate temperature overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid). The membranes were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was performed using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA), and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA).

3) Results

3.1) Animal Characteristics

Food intake was similar in the control vehicle, budesonide- and prednisone - treated animals (Table 5). Despite this, weight gain was lower ($p < 0.05$) in the budesonide than in the prednisone or in the control group. The body weight gain in rats given budesonide 0.75 and 1.0 mg/kg was similar to controls (data not shown). The percentage of weight gain (g/day) per food intake (g/day) was lower in the budesonide than in the control group, and it was higher in the prednisone than in the control or budesonide group.

The mean weight of the intestine (mg/cm length) and the percentage of the intestinal wall comprised of mucosa were similar in the control, prednisone and budesonide groups (Table 6). Accordingly, the rates of sugar uptake were expressed as nmol of substrate taken up per 100 mg dry weight of intestinal tissue per minute (nmol*100 mg tissue^{-1}*min^{-1}).

3.2 Uptake of Sugars

A curvilinear relationship was noted between the concentration of D-glucose (4-64 mM) and the rate of glucose uptake (data not shown). The estimated values of the maximal transport rate (Vmax) and of the apparent Michaelis constant (Km) for glucose uptake were unaffected by treatment with prednisone or with budesonide (0.25 mg/kg) (Table 7). Budesonide given at a dose of 1.0 mg/kg also had no effect on D-glucose uptake (data not shown). The jejunal and ileal rates of uptake of L-glucose and of D-mannitol were unaffected by prednisone or budesonide, as compared with the control group (Table 8).

A linear relationship was noted between increasing concentrations (4-64 mM) and the rate of uptake of fructose (data not shown). Because this relationship was linear over the concentrations studied, it was not possible to calculate values for Vmax or for Km. In the jejunum and ileum, the value of the slope of this linear relationship was higher ($p<0.05$) in the prednisone and in the budesonide groups, as compared with the control group (Table 9). A dose of budesonide of 1 mg/kg also increased ($p<0.05$) the slope of fructose uptake into the jejunum to 13.9 nmol*100 mg tissue^{-1}*min^{-1}, as compared with 12.1 nmol*100 mg tissue^{-1}*min^{-1} in the control group.

3.3) Transporter Protein Abundance and Expression of mRNA

In animals given prednisone the SGLT1 abundance was reduced ($p<0.05$) in the jejunum as compared with the control group, and did not change in the ileum (Table 10). Budesonide did not affect the abundance of SGLT1. The Na^+/K^+-ATPase alpha 1 was not changed in either the jejunum or ileum (Table 10). No changes in Na^+/K^+-ATPase beta 1 abundance were observed in the jejunum. However, the Na^+/K^+-ATPase beta 1 abundance was reduced in the ileum of animals given prednisone, as compared to animals given control vehicle or given budesonide. Steroids had no effect on GLUT5 and GLUT2 abundance in the jejunum or ileum.

No differences in mRNA expression of SGLT1, Na^+/K^+-ATPase alpha 1 or Na^+/K^+-ATPase beta 1 were observed in the jejunum and ileum of animals given budesonide or prednisone, as compared with the control group (Table 11). Steroids had no effect on GLUT5 or GLUT2 mRNA expression in the jejunum or ileum.

3.4) Early Response Gene, Proglucagon and Ornithine Decarboxylase (ODC) mRNA Expression

No detectable signal was observed for c-fos. Steroids had no effect on the expression of c-myc and c-jun (Table 12). Steroids had no effect on proglucagon mRNA expression at either site. In the ileum but not in the jejunum of animals given prednisone, the ODC mRNA expression was increased as compared to animals in the control group or those given budesonide.

3.5) Cytokine Gene Expression

Steroids had no effect on the mRNA expression of TNF-α, IL-2, IL-6 or IL-10 in either the jejunum or ileum (Table 13).

4. Discussion

Animals fed budesonide had a reduced rate of weight gain which was not explained by a lower food intake, or by a lower rate of intestinal uptake of glucose or fructose. In fact, animals given budesonide had increased uptake of fructose (Table 9). The mechanism responsible for this lower weight gain in rats given budesonide was not established in this study. However, it is possible that the effect of budesonide on weight gain may have been spurious, firstly because at higher doses (0.75 and 1.0 mg/kg) weight gain was similar to controls, and secondly because budesonide (0.25 mg/kg) had no effect on body weight gain in animals fed a semisynthetic diet enriched with saturated or polyunsaturated fatty acids (Table 18). It is interesting to note that the dose of prednisone used in this study did not alter food intake or body weight gain, despite its systemic nature.

Prednisone acts systemically on the intestine, in contrast to the largely local action of budesonide [Brattsand, 1990]. In adult animals, prednisone (in a dose of 0.75 mg/kg for 28 days) increases glucose absorption [Batt and Scott, 1982]. The lack of effect of prednisone on glucose uptake in this study may be due to the younger age of the animals. The lack of effect of prednisone or budesonide on the jejunal or ileal uptake of L-glucose or D-mannitol (Table 8) suggests that the passive paracellular contribution to sugar uptake is also unaffected by these steroids. The lack of effect of either prednisone or budesonide on the value of the Vmax of glucose uptake in these four week post-weanling rats (Table 7) suggests that there was no change in the activity of the sodium-dependent glucose transporter in the brush border membrane, SGLT1. The reduced jejunal abundance on SGLT1 in animals given prednisone (Table 10) did not affect the activity of the transporter. This suggests that under some conditions there may be a dissociation between SGLT1 protein abundance and transporter activity.

The linear relationship between fructose uptake and concentration (over the range used in this study) precluded the calculation of values for Vmax or Km. Fructose uptake is mediated by GLUT5 (the sodium-independent fructose transporter in the brush border membrane). In this study the increased fructose uptake with budesonide or prednisone was not associated with enhancement in the abundance of GLUT5 protein or expression of GLUT5 mRNA. This suggests that the increase in fructose uptake observed with steroids is due to post-translational control of GLUT5. Another possibility would involve the distribution of GLUT5 along the crypt-villus unit that could be altered without changes in the total abundance of GLUT5, as measured by Western blotting [Thiesen et al., 2001]. GLUT2 transports fructose across the basolateral membrane, and recent evidence suggests that GLUT2 may also be in the BBM [Kellet and Helliwell, 2000; Helliwell et al., 2000a; Helliwell et al., 2000b]. However, no changes in GLUT2 protein abundance or mRNA expression were observed, so that it is unlikely that the increased fructose uptake observed with steroids could be explained by enhanced transport of this sugar out of the enterocyte.

Steroids have been suggested to increase the expression of a series of transcription factors [Baxter, 1976; Thiele et al., 1999; Nosti-Escanilla and Pena, 1998, Neurath et al., 1998]. Early response genes such as c-myc, c-jun and c-fos have been demonstrated to be involved in processes of proliferation and differentiation, as well as ODC, a key enzyme in the synthesis of polyamines and a requirement in any proliferative event [Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and

McBurney, 1998]. Proglucagon has been shown to be involved in the intestinal adaptive process [Mojsov et al., 1986; Orskov et al., 1987; Tappenden and McBurney, 1998]. For example, short chain fatty acids increase the ileal c-myc and proglucagon expression in rats undergoing intestinal resection [Tappenden and McBUrney, 1998]. The finding in this study of increased ileal ODC mRNA with prednisone (Table 8) may explain part of the enhanced fructose uptake with this steroid. ODC, may be responsible for the increased uptake of D-fructose in animals given prednisone. By a mechanism probably involving proliferative events, ODC might be able to induce transporters such as GLUT5 and consequently absorption. Clearly, there must be other signals responsible for the adaptive effect of steroids on intestinal fructose uptake.

The administration of IL-6, IL-1α and IL-8 has been show to increase the uptake of glucose *in vitro* studies [Hardin et al., 2000]. It was hypothesized that changes in cytokine expression might be responsible for the phenotypic alterations in transport activity and absorption acting by intracellular signalling mechanisms that would result in expression of transporters [Hardin et al., 2000; Berridge and Tan, 1995; Taylor et al., 1992]. However, cytokine signalling was not observed with either prednisone or budesonide. Therefore, the effect of steroids on the fructose uptake was not explained by alterations in the mRNA expression of TNF-α, IL-2, IL-6 and IL-10.

In summary, 1) giving post- weaning rats four weeks of budesonide or prednisone in doses equivalent to those used in clinical practice increases fructose but not glucose uptake; and 2) the enhanced uptake of fructose was likely regulated by post-transcriptional events.

Table 5: Food Intake and Body Weight Gain.

	Food Intake (g/day)	**Weight Gain (g/day)**	**Weight Gain per Food Intake, %**
Control Vehicle	22.3 ± 0.8	9.1 ± 0.2	40.8 ± 0.4
Prednisone	21.1 ± 1.1	9.0 ± 0.2	42.6 ± 0.7*
Budesonide	21.0 ± 0.8	8.2 ± 0.3*	39.0 ± 0.4*#

Mean ± SEM. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$, budesonide or prednisone vs control vehicle.

#, $p<0.05$, budesonide vs prednisone.

Table 6: Characteristics of Intestine.

	Intestinal Weight (mg/cm)	**% of Intestinal Wall Comprised of Mucosa**
Jejunum		
Control Vehicle	20.0 ± 0.7	53.4 ± 3.2
Prednisone	22.6 ± 2.1	45.7 ± 6.0
Budesonide	18.3 ± 1.2	53.3 ± 3.6
Ileum		
Control Vehicle	15.4 ± 2.8	47.6 ± 6.1
Prednisone	13.5 ± 1.1	37.5 ± 6.2
Budesonide	10.7 ± 0.7	38.9 ± 6.4

Mean ± SEM. The dose of budesonide was 0.25 mg/kg, and the prednisone dose was 0.75 mg/kg.

Values were not significantly different.

Table 7: Kinetic Constants of Intestinal Uptake of D-Glucose.

	Maximal Transport Rate **Vmax (nmol.100 mg-1.min-1)**	**Apparent Michaelis Constant** **Km (mM)**
Jejunum		
Control Vehicle	1492 ± 84	47 ± 5
Prednisone	1366 ± 150	43 ± 9
Budesonide	1468 ± 58	44 ± 3
Ileum		
Control Vehicle	1652 ± 272	80 ± 21
Prednisone	1659 ± 382	85 ± 30
Budesonide	1808 ± 373	82 ± 26

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1}*min^{-1}. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

Values were not significantly different.

Table 8: Rates of Uptake of L-glucose and D-mannitol.

	L-Glucose	D-Mannitol
Jejunum		
Control Vehicle	14 ± 1	17 ± 1
Prednisone	16 ± 2	18 ± 1
Budesonide	14 ± 1	16 ± 1
Ileum		
Control Vehicle	10 ± 1	12 ± 1
Prednisone	10 ± 1	14 ± 2
Budesonide	10 ± 1	13 ± 1

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1}*min^{-1}* mM^{-1}.

The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

Values were not significantly different.

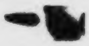
Table 9: Slopes of Relationship Between Concentration and Rates of Uptake of D-Fructose in the Jejunum and Ileum

	Jejunum **Slope**	**Ileum** **Slope**
Control Vehicle	12.1 ± 0.3	10.1 ± 0.3
Prednisone	13.2 ± 0.1*	11.7 ± 0.2*
Budesonide	12.8 ± 0.1*	12.1 ± 0.8*

Mean ± SEM. The values of the slopes are expressed as nmol*100 mg tissue^{-1}*min^{-1}. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$, budesonide or prednisone vs control vehicle.

Table 10: Protein Abundance Related to D-glucose and Fructose Uptake.

	SGLT1	Na^+/K^+ ATPase α1	Na^+/K^+ ATPase β1	GLUT5	GLUT2
					
	P B	P B C	P B C	P B	P B C
Jejunum					
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	0.47 ± 0.29*	1.13 ± 0.14	1.13 ± 0.13	1.13 ± 0.75	1.02 ± 0.31
Budesonide	0.86 ± 0.53	1.24 ± 0.40	1.00 ± 0.27	0.93 ± 0.37	1.09 ± 0.12
	P B C	P B C	P B C	P B C	P B C
Ileum					
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.16 ± 0.36	1.53 ± 0.37	0.73 ±	0.98 ± 0.33	1.30 ± 0.55
Budesonide	0.86 ± 0.35	0.94 ± 0.63	0.99 ± 0.24	0.91 ± 0.37	1.39 ± 0.48

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg. There were at least three animals in each group.

P = Prednisone.

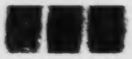
B = Budesonide.

C = Control.

*, $p<0.05$, prednisone vs control vehicle.

#, $p<0.05$, budesonide vs prednisone.

Table 11: mRNA Expression Related to D-glucose and Fructose Uptake.

	SGLT1	**Na$^+$/K$^+$ ATPase α1**	**Na$^+$/K$^+$ ATPase β1**	**GLUT5**	**GLUT2**
	P B C	P B C	P B C	P B C	P B C
Jejunum					
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	0.69 ± 0.69	0.79 ± 0.42	0.57 ± 0.42	0.83 ± 0.28	1.01 ± 0.26
Budesonide	1.21 ± 0.48	2.15 ± 1.81	1.23 ± 0.87	0.83 ± 0.44	1.23 ± 0.65
					
	P B C	P B C	P B C	P B C	P B C
Ileum					
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.47 ± 0.45	1.20 ± 0.59	1.41 ± 0.75	1.41 ± 0.41	1.62± 0.93
Budesonide	1.32 ± 0.55	1.29 ± 0.41	2.58 ± 3.41	0.56 ± 0.44	1.10 ± 0.27

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg. There were at least three animals in each group.

P = Prednisone.

B = Budesonide.

C = Control.

Values were not significantly different.

Table 12: mRNA Expression of Early Response Genes, Ornithine Decarboxylase (ODC) and Proglucagon.

	c-jun	c-myc	c-fos	Proglucagon	ODC
Jejunum					
Control	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0
Prednisone	0.97 ± 1.04	1.02 ± 0.99	N.S.	1.06 ± 0.13	0.93 ± 0.25
Budesonide	1.14 ± 0.55	1.05 ± 0.47	N.S.	0.64 ± 0.72	1.21 ± 0.26
Ileum					
Control	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0
Prednisone	1.58 ± 0.67	1.40 ± 0.34	N.S.	1.40 ± 0.55	1.17 ± 0.17*#
Budesonide	1.16 ± 0.39	1.16 ± 0.32	N.S.	0.86 ± 0.45	0.96 ± 0.06

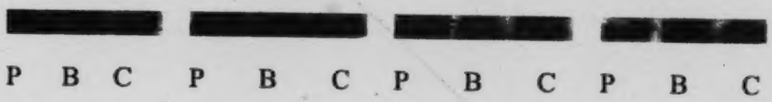
Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg. There were at least three animals in each group.

N. S. = No signal.

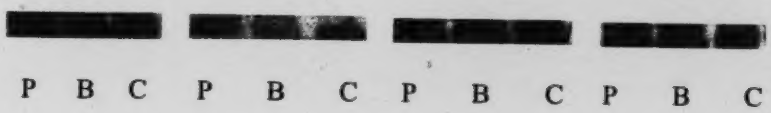
*, $p<0.05$, prednisone vs control vehicle.

#, $p<0.05$, prednisone vs budesonide.

Table 13: Cytokine Gene Expression



Jejunum	TNF-α	IL-2	IL-6	IL-10
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.32 ± 0.30	1.22 ± 0.55	1.23 ± 0.26	1.43 ± 0.40
Budesonide	1.41 ± 0.39	1.39 ± 0.43	1.25 ± 0.20	1.43 ± 0.36



Ileum	TNF-α	IL-2	IL-6	IL-10
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.42 ± 0.57	1.01 ± 0.19	1.20 ± 0.26	1.25 ± 0.33
Budesonide	1.25 ± 0.75	0.68 ± 0.17	1.02 ± 0.30	0.80 ± 0.11

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg. There were at least three animals in each group.

P = Prednisone.

B = Budesonide.

C = Control.

Values were not significantly different.

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G) LOCALLY AND SYSTEMICALLY ACTIVE GLUCOCORTICOIDS MODIFY INTESTINAL ABSORPTION OF LIPIDS IN RAT

1) Introduction

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis [Lofberg et al., 1993; Nyman-Pantelidis et al., 1994; Tarpila et al., 1994; Girdwood and Petrie, 1987; Murphy et al., 1982; Batt and Peters, 1976; Wright, 1993] . However, the systemically active steroids may be associated with potentially serious adverse effects [Lofberg et al., 1993; Cann and Holdsworth, 1987; Reshef et al., 1992; Rutgeerts et al., 1994]. Even topical treatment with steroids using retention enemas or foams depresses the plasma cortisol concentration [Cann and Holdsworth, 1987; Reshef et al., 1992]. The high prevalence of these adverse effects has been a major impetus for the development of non-systemic steroids. Budesonide is a non-systemic steroid with high topical activity, low systemic bioavailability, and rapid first pass metabolism [Brattsand, 1990; Thiesen and Thomson, 1996]. Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease [Brignola et al., 1992; Brignola et al., 1994; Greenberg et al., 1994; Danielsson et al., 1992].

In young animals steroids induce precocious development of some of the intestinal brush border membrane enzymes, and facilitate the induction of specific enzymes by dietary carbohydrate [Haynes and Murad, 1985; Lebenthal et al., 1972; Batt and Peters, 1976]. Systemically active steroids given by mouth enhance glucose uptake by adult animals [Batt and Scott, 1992], but their effect on lipid uptake is unknown.

Dexamethasone (128 mg/kg/day, given subcutaneously for seven days) blunts the expected adaptive response following intestinal resection [Park et al., 1994].

Proglucagon-derived peptides originate from processing and cleavage of the proglucagon gene product[Mojsov et al., 1986; Orskov et al., 1987] in the L-cells present in the ileum and colon [Larsson et al., 1975]. It has been suggested that the mRNA levels of proglucagon and ornithine decarboxylase (ODC) as well as the early response genes (ERG) such as c-myc, c-jun and c-fos are involved in the adaptive process of the intestine such as small intestinal resection [Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992, Tappenden and McBurney, 1998]. It is unknown if proglucagon, ODC or ERGs in the intestine are influenced by steroids.

Intestinal lipid uptake occurs by a process of passive permeation, but a component of the uptake of long-chain fatty acids is also mediated by the sodium/hydrogen exchanger and/or by fatty acid binding proteins in the brush border membrane such as the fatty acid binding protein ($FABP_m$) and the fatty acid transporter (FAT), or in the cytosol of the enterocyte such as the liver fatty acid binding protein (L-FABP) and the ileal lipid binding protein (ILBP) [Schoeller et al., 1995; Poirier et al., 1996; Schoeller et al., 1995; Keelan et al., 1996; Niot et al., 1997]. It is unknown if the intestinal fatty acid binding proteins are influenced by steroids.

A wide variety of cytokines are produced locally by the intestinal epithelium, and they are essential for the homeostasis of the intestinal tissue during development [Fiochi, 1997; Kagnoff et al., 1996]. Cytokine gene expression has been shown to be regulated by hydrocortisone and dexamethasone during postnatal small intestinal development [Schaeffer et al., 2000]. Cytokines have been shown to alter nutrient absorption. For instance, interleukins IL-6, IL-1α , and IL-8 have been shown to increase glucose absorption and IL-1α and IL-8 have been shown to increase L-proline absorption. Furthermore, the abundance of SGLT1 was not changed by cytokines [Hardin et al., 2000]. It is not known if the cytokines in the intestine are influenced by prednisone or budesonide.

Accordingly, this study was undertaken to assess the influence of budesonide and prednisone, in doses equivalent to those used in clinical practice, on the intestinal uptake of lipids in young growing rats, as well as the effect of these steroids on the mRNA expression of several signals of adaptation including proglucagon, ODC, ERGs (c-myc, c-fos and c-jun), enterocyte cytosolic lipid binding proteins and cytokines in young growing rats. The results show that budesonide and prednisone enhance the jejunal and ileal uptake of some lipids, by a process that does not appear to involve alterations in the expression of any of these signals, or either of these lipid binding proteins.

2) Methods and Materials

2.1) RNA blotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E.coli*) were transformed with plasmids containing the desired DNA sequences to be used as a probe for the Northern blotting. ERG probes were obtained from Oncogene Research Products; cDNA probe encoding proglucagon was obtained from Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; ODC probe was obtained from Dr. Blackshear, University of Chicago; L-FABP and ILBP probes were provided by Dr. Agellon, University of Alberta; and TNF-α, IL-2, IL-6 and IL-10 were obtained from BIO/CAN Scientific. A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA).

RNA was extracted from the jejunum and ileum of at least three animals in each group. These segments were homogenized in a denaturing solution, containing guanidinium thiocyanate, using the biorad fast prep shaking centrifuge. Following addition of 2 M sodium acetate, a phenol cholorform extraction was done. The upper aqueous phase was transferred to a tube and the RNA was precipitated with isopropanol and washed with 70% ethanol.

Total RNA was separated based on molecular weight as it was electrophoresed through denaturing agarose gel (1.16% agarose). RNA was then transferred from the gel to a nylon membrane by capillary action overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA onto it. As a pre-hybridization, membranes were incubated during 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at the adequate hybridization temperature overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid) in order to reduce non-specific binding. They were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was done using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA) and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life

Science Group, Cleveland, Ohio, USA). To determine the exact RNA quantity that has been loaded, the 28S ribosomal band density on the membrane was evaluated from the blot itself after the transfer.

3) Results

3.1) Animal Characteristics

Food intake was similar in the control, budesonide- and prednisone - treated animals (Table 5). Despite this, weight gain was lower ($p < 0.05$) in the budesonide than in the prednisone or in the control vehicle group. This was likely a spurious result, since the body weight gain in rats given budesonide 0.75 and 1.0 mg/kg was similar to controls (data not shown). The percentage of weight gain (g/day) per food intake (g/day) was lower in the budesonide than in the control vehicle group, was higher in the prednisone than in the control group, and was also higher in the prednisone than in the budesonide group.

The mean weight of the intestine (mg/cm length) and the percentage of the intestinal wall comprised of scrapable mucosa were similar in the control vehicle, prednisone and budesonide groups (Table 6). Accordingly, the rates of uptake were expressed as nmol of substrate taken up per 100 mg dry weight of intestinal tissue per minute (nmol*100 mg tissue^{-1}*min^{-1}*mM for the lipids).

3.2) Uptake of Lipids

As compared with the control group, prednisone increased the jejunal uptake of cholesterol as well as the ileal uptake of lauric (12:0), palmitic (16:0), linoleic (18:2) and linolenic (18:3) acids (Table 14). Budesonide (0.25 mg/kg) increased the jejunal uptake of oleic acid (18:1), and increased ileal uptake of linoleic acid (18:2). With budesonide as compared with prednisone, the jejunal uptake of 18:1 was higher whereas cholesterol was lower, and ileal uptake of 18:3 was lower. Budesonide in doses of 0.75 and 1.0 mg/kg also had no effect on the jejunal or ileal uptake of 16:0, 18:2 or cholesterol (data not shown).

3.3) mRNA Expression

Steroids had no effect on the mRNA expression in the jejunum or ileum of c-myc or c-jun (Table 15). No detectable signal was observe for c-fos.

Steroids had no effect on proglucagon mRNA expression at either site (Table 16). In the ileum but not the jejunum of animals given Pred but not Bud, the ODC mRNA expression was increased as compared to animals in the control vehicle group or given budesonide.

Neither prednisone nor budesonide altered the mRNA expression of the liver fatty acid binding protein (L-FABP) in the jejunum or ileum (Table 17). As expected, the ileal lipid binding protein (ILBP) mRNA was present only in the ileum, and its abundance in this site was also unaffected by prednisone or budesonide.

Steroids had no effect on the mRNA expression of TNF-α, IL-2, IL-6 or IL-10 in either the jejunum or ileum (Table 13).

4) Discussion

Animals given budesonide had a slightly reduced rate of weight gain which was not explained by a lower food intake (Table 5), or by a lower rate of intestinal uptake of lipids (Table 14). In fact, animals given budesonide had increased jejunal uptake of 18:1 and ileal uptake of 18:2. The mechanisms responsible for this lower weight gain in rats given budesonide were not established in this study. However, this effect may have been spurious, because at higher doses of budesonide (1.0 mg/kg), weight gain was similar to controls (data not shown). It is interesting to note that the dose of prednisone used in this study did not alter food intake or body weight gain, despite its systemic nature.

Intestinal lipid uptake occurs by a process of passive permeation, but a component of the uptake of long-chain fatty acids is also mediated by the sodium/hydrogen exchanger and/or by fatty acid binding proteins in the brush border membrane such as ($FABP_m$) and (FAT), or in the cytosol of the enterocyte such as the L-FABP and the ILBP [Schoeller et al., 1995; Poirier et al., 1996; Schoeller et al., 1995; Keelan et al., 1996; Niot et al., 1997]. The increased jejunal and ileal uptake of lipids in animals given budesonide or prednisone (Table 14) does not distinguish between which of these passive or mediated steps may have been affected by steroids. The enhanced uptake of lipids as a result of giving budesonide or prednisone was not explained by alterations in the animal's food intake or mucosal mass (Tables 5 and 6). Adaptations in lipid uptake may be due to alterations in the lipid content of the brush border membrane (BBM) [Keelan et al., 1996; Deren et al., 1967]. Such measurements were not performed in this study. However, any steroid-associated change in the lipophilic properties of the BBM would be expected to modify the uptake of all lipids, and this did not occur (Table 14).

The role of the lipid binding proteins in the enterocyte in the overall control of lipid absorption remains to be determined [Niot et al., 1997; Drozdowski et al., 2001]. In this study, there were steroid-associated alterations in fatty acid and cholesterol uptake in the jejunum and ileum of rats given prednisone or budesonide (Table 14), without any demonstrable alteration in the expression of the mRNAs for either L-FABP or ILBP

(Table 17). We did not assess the expression of the mRNAs for fatty acid transporters in the BBM or for I-FABP in the cytosol, so we do not know if a change occurred in some other protein-mediated component important in the changes in lipid uptake. Also, we did not assess the abundance of L-FABP , ILBP, $FABP_m$ or FAT proteins in the enterocytes of animals given steroids. Thus, it is still possible that these or other lipid binding proteins do play a role in the steroid-associated alterations in lipid uptake, but that the changes in binding protein abundance were not reflected by alterations of mRNA expression for the L-FABP and ILBP. Recent support for a dissociation between lipid uptake and lipid binding proteins comes from the finding that clones of mice with disrupted genes for the intestinal fatty acid binding protein (I-FABP) are viable, have elevated plasma triacylglycerol, and weigh more. It is appreciated that this finding illustrates the effects of another lipid binding protein, I-FABP, which was not analysed in this study [Vassielva et al., 2001].

Proglucagon-derived peptides originate from processing and breakage of the proglucagon gene products [Mojsov et al., 1986;Orskov et al., 1987] in the L-cells present in the ileum and colon [Larsson et al., 1975]. Proglucagon and ornithine decarboxylase (ODC) as well as the early response genes (ERG) such as c-myc, c-jun and c-fos mRNA expression are involved in the adaptive process of the intestine [Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden et al., 1998]. Among the possible signals involved in this steroid-associated increase in lipid uptake, only the mRNA for ODC changed. It is well known that ODC is a key enzyme in the synthesis of polyamines, and that these are a requirement in any proliferative event. It is possible that an enhancement of the expression of this enzyme could result in the phenotypic changes observed in this study. This finding does not discard the possible influence of these other signals (ERG, proglucagon and cytokines) in this adaptive process, since there may be post-transcriptional regulatory mechanisms involved.

A wide variety of cytokines are produced locally by the intestinal epithelium, and they are essential for the homeostasis of the intestinal tissue during development [Fiochi, 1997; Kagnoff et al., 1996]. Cytokine gene expression has been shown to be regulated by hydrocortisone and dexamethasone during postnatal small intestinal development [Schaeffer et al., 2000]. Cytokines also change nutrient transport absorption. For instance, interleukins IL-6, IL-1α , and IL-8 have been shown to increase glucose absorption and IL-1α and IL-8 have been shown to increase L-proline absorption [Hardin et al., 2000]. In order to analyse the expression of some inflammatory cytokines, the mRNA expression for TNF-α, IL-2 and IL-6 was observed, and to analyse the expression of a cytokine with anti-inflammatory properties, the mRNA for IL-10 was observed. It was initially speculated that due to the anti-inflammatory activity of glucocorticosteroids, the mRNA expression of TNF-α, IL-2 and IL-6 would be decreased. In fact, no changes in the cytokine expression were observed. Therefore, glucocorticosteroids do not change the mRNA expression of these cytokines in the intestine under these experimental conditions, and the anti-inflammatory activity of glucocorticosteroids might involve down-regulation of TNF-α, IL-2 and IL-6 abundance, a parameter not measured in this study.

In summary, giving weanling rats four weeks of oral budesonide or prednisone in doses equivalent to those used in clinical practice enhances the uptake of some lipids. These changes are not explained by variations in the animal's food intake or in the weight of the mucosa, and were not associated with alterations in the expression of the mRNAs of ERGs, proglucagon, lipid binding proteins L-FABP and ILBP and cytokines, but ODC may be implicated in this adaptive process. The broader effect of prednisone than budesonide on lipid uptake raises the possibility that the mechanism was related to some systemic effect. The implication of these findings to humans remains to be established.

Table 14: Rates of Uptake of Fatty Acids and Cholesterol

Drug	Control Vehicle	Prednisone	Budesonide
Jejunum			
12:0	64 ± 11.9	86 ± 16.6	70.8 ± 11.8
16:0	9.5 ± 0.9	9.7 ± 1.1	10.9 ± 1.5
18:0	13.7 ± 2.1	12.1 ± 1.6	8.9 ± 0.9
18:1	8.8 ± 0.6	9.2 ± 0.7	18.0 ± 1.2*#
18:2	7.3 ± 1.0	10 ± 0.9	7.7 ± 0.8
18:3	11.1 ± 0.8	13.5 ± 2.1	11.6 ± 1.0
Cholesterol	4.4 ± 0.6	10.6 ± 2.2*	5.4 ± 1#
Ileum			
12:0	49.8 ± 10.6	114 ± 5.7*	87.3 ± 14.1
16:0	4.2 ± 0.6	9.7 ± 1.5*	6.7 ± 0.9
18:0	7.3 ± 0.8	8.9 ± 1.0	8.5 ± 0.9
18:1	5.7 ± 1.1	6.3 ± 0.8	5.4 ± 0.5
18:2	5.5 ± 0.9	12.2 ± 0.9*	11.0 ± 0.8*
18:3	7.3 ± 1.2	12.1 ± 1.2*	8.5 ± 0.7#
Cholesterol	3.2 ± 0.4	4.2 ± 0.6	3.8 ± 0.4

Mean ± SEM. The rates of uptake of fatty acids are expressed as nmol * 100 mg tissue^{-1} *min^{-1}* mM^{-1}. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$, budesonide or prednisone vs control vehicle

#, $p<0.05$, budesonide vs prednisone

Table 15: Early Response Gene mRNA Expression.

	c-jun	**c-myc**	**c-fos**
	n = 4	n = 5	
Jejunum			
	P B C	P B C	
Control	1 ± 0	1 ± 0	N.S.
Prednisone	0.97 ± 1.04	1.02 ± 0.99	N.S.
Budesonide	1.14 ± 0.55	1.05 ± 0.47	N.S.
	n = 4	n = 4	
Ileum			
	P B C	P B C	
Control	1 ± 0	1 ± 0	N.S.
Prednisone	1.58 ± 0.67	1.40 ± 0.34	N.S.
Budesonide	1.16 ± 0.39	1.16 ± 0.32	N.S.

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

The values were expressed as arbitrary units in relation to control

N. S. = No signal

P = Prednisone

B = Budesonide

C = Control

n = number of animals

None of these differences was statistically significant.

Table 16: Ornithine decarboxylase (ODC) and Proglucagon Gene Expression.

	ODC	Proglucagon
	n = 5	n = 3
Jejunum	P B C	P B C
Control	1 ± 0	1 ± 0
Prednisone	0.93 ± 0.25	1.06 ± 0.13
Budesonide	1.21 ± 0.26	0.64 ± 0.72
	n = 5	n = 3
Ileum	P B C	P B C
Control	1 ± 0	1 ± 0
Prednisone	1.17 ± 0.16*	1.40 ± 0.55
Budesonide	0.96 ± 0.06#	0.86 ± 0.45

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

The values were expressed as arbitrary units in relation to control

P = Prednisone

B = Budesonide

C = Control

n = number of animals

*, $p<0.05$, budesonide or prednisone vs control.

#, $p<0.05$, budesonide vs prednisone.

Table 17 : mRNA Expression of L-FABP and ILBP.

Drug	**L-FABP**	**ILBP**
	n = 3	
Jejunum		
Control Vehicle	1 ± 0	N.S.
Prednisone	1.05 ± 0.16	N.S.
Budesonide	1.53 ± 0.40	N.S.
	n = 3	n = 3
Ileum		
Control Vehicle	1 ± 0	1 ± 0
Prednisone	1.63 ± 0.97	1.28± 0.61
Budesonide	1.43 ± 0.61	1.30 ± 0.53

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

The values were expressed as arbitrary units in relation to control

N.S. = no signal

L-FABP,	liver fatty acid binding protein
ILBP,	ileal lipid binding protein

n = number of animals

None of these differences was statistically significant.

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H) THE STIMULATING EFFECT OF LOCALLY AND SYSTEMICALLY ACTING GLUCOCORTICOSTEROIDS ON INTESTINAL FRUCTOSE TRANSPORT IN RATS IS INCREASED BY FEEDING A SATURATED FATTY ACID DIET

1) Introduction

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis [Brattsand, 1990; Brignola et al., 1992; Brignola et al., 1994; Danielson and Prytz, 1994; Lofberg et al., 1993; Nyman-Pantelidis et al., 1984; Reshef et al., 1992; Ehrich et al., 1992; Greenberg, 1994]. However, systemic steroids may be associated with numerous and potentially serious adverse effects [Brattsand, 1990; Girdwood and Petrie, 1987; Haynes and Murad, 1985; Danielsson et al., 1992]. Even topical treatment with steroids using retention enemas or foams depresses the plasma cortisol concentration [Reshef et al., 1992, Thiesen et al., 1996; Tarpila et al., 1994]. For this reason, non-systemic steroids have been developed. The non-systemic steroid budesonide has high topical activity, low systemic bioavailability, and rapid first pass metabolism in the intestine and liver [Brattsand, 1990]. Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease [Haynes and Murad, 1985].

Intestinal adaptation is a process that occurs in response to physiological or pathological processes such as intestinal resection, aging, diabetes, abdominal radiation, chronic alcohol intake, and feeding diets of varying lipid, protein or carbohydrate composition [Thomson et al., 1990; Thomson and Wild, 1997]. Thus, there are different morphological and physiological characteristics of the intestine depending upon the nutrient composition of the diet [Thomson and Wild, 1997; Diamond and Karasov; 1984; Thomson and Rajotte, 1983; Thomson and Rajotte, 1983b]. In adult rats, the intestinal uptake of sugars and lipids is enhanced by feeding an isocaloric semisynthetic diet enriched with saturated as compared with polyunsaturated fatty acids [Thomson, 1982].

In young animals, steroids induce precocious development of the intestinal brush border membrane (BBM) enzymes, and facilitate the induction of several BBM enzymes by dietary carbohydrate (Lebenthal et al., 1972; Batt and Peters, 1976]. Steroids given by mouth enhance glucose absorption by adult animals [Batt and Peters, 1975; Batt and Scott, 1982]. Dexamethasone given subcutaneously blunts the expected adaptive response following intestinal resection [Park et al., 1994].

The Na^+ -gradient across the BBM provides the driving force for the glucose transport [Ricklis and Quastel, 1958]. This gradient is maintained by the action of the Na^+/K^+-ATPase which is restricted to the basolateral membrane [Hirayama et al., 1992]. SGLT1 mediates the BBM Na^+/glucose cotransport [Vehyl et al., 1992; Vehyl et al., 1993; Weber et al., 1991]. Fructose uptake across the BBM is mediated by facilitated diffusion by GLUT5 [Burant et al., 1992; Rand et al., 1993; Shu et al., 1997], whereas GLUT2 mediates the facilitative Na^+-independent diffusion of glucose, galactose and fructose through the BLM [Burant et al., 1992; Caspary et al., 1968]. Recently, GLUT2 has been described to be present in the BBM [Kellet and Heliwell, 2000; Heliwell et al., 2000a; Heliwell et al., 2000 b; Kellet, 2001].

Proglucagon-derived peptides originate from processing and breakage of the proglucagon gene [Mojsov et al., 1986; Orskov et al., 1987] in the L-cells present in the ileum and colon [Larsson et al., 1975]. It has been suggested that the mRNA levels of proglucagon and ornithine decarboxylase (ODC) as well as the mRNAs of early response genes such as c-myc, c-jun and c-fos may be involved in the intestinal adaptive process of the intestine ([Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998].

The adaptive effect of locally or systemically acting steroids on intestinal nutrient transport associated with changes in dietary lipids has not been described. We formulated the hypothesis that feeding weaning rats an isocaloric semisynthetic saturated as compared with a polyunsaturated diet will enhance further the increased intestinal uptake of sugars observed when animals are given steroids. The results show that 1) prednisone and budesonide given in doses used clinically in humans enhance the intestinal uptake of fructose but not glucose; 2) this enhancement in fructose uptake can be increased further by feeding a diet enriched with saturated as compared with polyunsaturated fatty acids; 3) this adaptation is likely regulated by post-transcriptional and post-translational processes; and 4) this effect of diets and steroids may be signalled by variations in the mRNA expression of early response genes and proglucagon.

2) Methods and Materials

2.1) Immunohistochemistry

To prepare the histological sections, the samples from 4 animals in each group were dehydrated, embedded in paraffin, sectioned in 4 to 5 micron sections and mounted on Aptex coated slides. The slides were then dried overnight at 37° C or dried at 65° C for 30 minutes. Following, the slides were deparaffinized by heating at 60° to 70° C for 10 minutes, 2) incubating in a xylene solution 3 times for 5 minutes each, 3) incubating in absolute ethanol 3 times for 2 minutes each, 4) incubating in 90% ethanol for 1 minute, 5) incubating in 70 % ethanol for 1 minute. To avoid and destroy any endogenous peroxidase activity the slides were incubated in hydrogen peroxide/methanol solution for 6 minutes. After rinsing well in tap water, the slides were counterstained with Harris Hematoxylin for about 30 seconds and again rinsed in warm to hot tap water. After this step, the slides were drained and air-dried onto a Loress pad. Rehydration with 0.01 M phosphate buffered saline (PBS) was performed and followed by 15 minute incubation with the blocking reagent (20% normal goat serum). After blocking, primary antisera incubation with SGLT1 at the 1/800 dilution or GLUT5 at the 1/500 dilution for 30 minutes was performed. Series of washes with PBS were performed for 5 minutes and followed by incubation with the LINK reagent (secondary antibody) for 20 minutes and incubation with the LABEL reagent (streptavidin biotin tertiary reagent) for another 20 minutes. New washes with PBS and finally incubation with the DAB chromogen reagent for 5 minutes were performed. The DAB solution contains 3,3'-diaminobenzidine that forms a brownish end product that is visible under microscopy. The density of the signal was determined by transmittance densitometry using a Image 1D imaging densitometer. The crypt-villus unit was divided in 5 equal parts and the density of the signals were quantified in each of these compartments. The villus tip was called number 1 and the crypts called number 5. All the values were corrected for background. The final results were expressed in OD * mm^2 .

3) Results

3.1) Animal Characteristics

Prednisone and budesonide had no effect on the animals' food intake or body weight gain (Table 18). In the control vehicle, prednisone and budesonide groups, the weight gain was lower in the rats fed SFA as compared with PUFA (for example, in those given the control vehicle and fed SFA, the weight gain was 6.8 ± 0.6 g/day, as compared with 8.8 ± 0.3 in those fed PUFA , $p<0.05$). Prednisone and budesonide had no effect on the total weight of the intestine, or on the percentage of the intestinal wall comprised of mucosa (Table 19). Steroids had no effect on the villous height of the jejunum or ileum of rats fed SFA, or of the jejunum of animals fed PUFA. In the ileum of rats fed PUFA, the villous height was lower in animals given budesonide as compared with prednisone (181 ± 7 and 314 ± 14 mm, respectively, $p< 0.05$). In the ileum of rats given budesonide, the height of the villi was higher in those fed SFA than PUFA (239 ± 32 and 181 ± 7 mm, respectively, $p<0.05$)

3.2) Uptake of Sugars

There was a curvilinear relationship between the concentration and the rate of uptake of glucose (data not shown). The values of the maximal transport rate (Vmax) and apparent Michaelis constant (Km) for glucose uptake were unaffected by giving prednisone or budesonide as compared with control vehicle (Table 20). The value of the Vmax for ileal uptake of glucose was higher in control, prednisone and budesonide treated animals fed SFA versus PUFA (Table 21). The rates of uptake of L-glucose and D-mannitol were similar, and were unaffected by prednisone or budesonide in animals fed either PUFA or SFA (Table 22).

The relationship between fructose concentration and uptake was linear (data not shown). As a result, it was not possible to calculate values of Vmax or Km for this sugar. In animals fed SFA, giving prednisone or budesonide increased the value of the slope of the linear relationship between fructose concentration and uptake, as compared with rats given control vehicle (Table 23). Budesonide increased the ileal uptake of fructose by approximately 15%, as compared with those fed SFA and given control vehicle. This enhancing effect of prednisone and budesonide on fructose uptake was not observed in animals fed PUFA. In rats fed SFA, the uptake of fructose was lower in the jejunum but higher in the ileum of those given budesonide as compared with prednisone. The slope of the linear relationship between fructose concentration and uptake into the jejunum or ileum was greater in animals fed SFA as compared with PUFA, whether they are given control vehicle, prednisone or budesonide (Table 24).

3.3) Western and Northern Blotting

Animals fed SFA and given control vehicle, prednisone or budesonide had neither jejunal nor ileal effects on the abundance of SGLT1, Na^+/K^+-ATPase alpha 1 or beta 1 subunits (Table 25). However, animals fed PUFA and given prednisone or budesonide had decreased ileal abundance of SGLT1. The animals fed PUFA and given prednisone had decreased jejunal abundance of Na^+/K^+-ATPase beta 1. The animals given budesonide had a reduction in the jejunal and ileal abundance of SGLT1 in those fed SFA, as compared to animals fed PUFA (Table 26). Also, the ileal abundance of Na^+/K^+-ATPase alpha 1 was less in the ileum of those rats given budesonide and fed SFA as compared with PUFA.

The jejunal expression of SGLT1 mRNA was increased in animals fed PUFA and given prednisone, as compared to those given control vehicle (1.37 ± 0.29 vs. 1.0 ± 0, $p < 0.05$). There were no differences observed in Na^+/K^+-ATPase alpha 1 or Na^+/K^+-ATPase beta 1 mRNA expression in the jejunum or ileum of animals given steroids and fed PUFA or SFA (Table 27). Steroids had no effect on the ileal SGLT1 mRNA expression in rats fed SFA or PUFA. Animals given prednisone or budesonide had reduced ileal SGLT1 mRNA expression in those fed SFA as compared to PUFA (Table 28). Furthermore, in rats given budesonide there was reduced expression of SGLT1 mRNA in those fed SFA as compared with PUFA. The prednisone group had decreased expression of Na^+/K^+-ATPase alpha 1 mRNA in the jejunum and ileum of those fed SFA as compared with PUFA. Na^+/K^+-ATPase beta 1 mRNA expression was increased in animals given control vehicle and fed SFA as compared with PUFA.

Steroids had no effect on the GLUT2 abundance in the jejunum or ileum of rats feed SFA or PUFA (Table 29). In rats fed SFA, prednisone increased the jejunal GLUT5 abundance as compared to the budesonide or control vehicle groups (1.36 ± 0.33 vs. 1.0 ± 0, $p<0.05$), and reduced the ileal GLUT5 abundance as compared to controls (0.84 ± 0.23 vs. 1.0 ± 0, $p<0.05$). Changing dietary lipids had no effect on the protein abundance of GLUT5 or GLUT2 (Table 30). Steroids had no effect on the mRNA expression of GLUT2 and GLUT5 (Table 31). The expression of GLUT5 mRNA was unchanged by altering the lipid content of the diet (Table 32). However, the jejunal GLUT2 mRNA expression was lower in animal given prednisone or budesonide and fed SFA, as compared with PUFA (0.72 ± 0.20 vs. 1.0 ± 0 and 0.64 ± 0.13 vs. 1.0 ± 0, respectively, $p < 0.05$).

3.4) Immunohistochemistry

The abundance of SGLT1 and GLUT5 did not vary along the length of the jejunal villus (Table 33). In animals given control vehicle, SGLT1 and GLUT5 were higher in most villous segments of rats fed SFA versus PUFA. Steroids had no effect on the abundance of SGLT1 of rats fed SFA or PUFA. In contrast, as compared with control vehicle, prednisone and budesonide increased GLUT5 in the jejunal villus of animals fed either SFA or PUFA.

3.5) Early Response Gene (ERG) mRNA Expression

Steroids had no effect on the mRNA expression of the early response genes (ERG) (Table 34). The c-myc mRNA expression did not change whether the animals were fed SFA or PUFA (Table 35). The c-jun mRNA expression was increased in the ileum of animals given control vehicle and fed SFA as compared to PUFA (2.14 ± 0.79 vs 1.0 ± 0, $p< 0.05$).

3.6) Proglucagon and Ornithine Decarboxylase mRNA expression

Steroids and dietary lipids had no effect on the mRNA expression of ornithine decarboxylase (ODC) (Tables 36 and 37). Prednisone increased the ileal proglucagon mRNA expression in animals fed PUFA (1.51 ± 0.13 vs. 1.0 ± 0, $p < 0.05$), whereas budesonide increased the jejunal proglucagon mRNA expression in those fed SFA (1.03 ± 0.02 vs. 1.0 ± 0, $p <0.05$). The ileal expression of proglucagon mRNA in animals given control vehicle was higher in those fed SFA versus PUFA (2.15 ± 0.35 vs. 1.0 ± 0, $p<0.05$). The jejunal expression of proglucagon mRNA was lower in animals given prednisone and fed SFA as compared with PUFA (0.12 ± 0.01 vs. 1.0 ± 0, $p<0.05$). None of the other differences among the groups was statistically significant.

4) Discussion

Glucose uptake is mediated by a sodium-dependent carrier in the brush border membrane (BBM), SGLT1 [Thomson et al., 1981], and by passive permeation through the paracellular route [Caspary et al., 2000]. In adult rats, feeding SFA increases the value of the Vmax for glucose uptake in the jejunum and ileum [Thomson, 1982]. In this study, this was observed only for the ileum in these young animals (Table 21). The reason for the lack of change in glucose uptake of the jejunum of young animals in response to feeding SFA was not established in this study. We suggest that this is not due to an age-associated lack of response of SGLT1 to dietary lipids, because the ileal uptake of glucose was greater in animals fed SFA as compared with PUFA (Table 21). Neither prednisone nor budesonide alter glucose uptake in young rats fed SFA or PUFA . The lack of change in glucose uptake was not due to an obscuring effect of alterations in the contribution of the passive component of sugar uptake, as measured with L-glucose or D-mannitol (Table 22). This suggests that while the SGLT1 in the ileum of young animals is capable of responding to the stimulus of changes in dietary lipids, it is not responsive to the effects of these steroids.

Fructose is transported by the sodium-independent transporter in the brush border membrane, GLUT5. Feeding PUFA prevented the enhanced uptake of fructose in animals fed SFA and given prednisone or budesonide (Tables 23 and 24). Thus, the effect of steroids appears to be specific for GLUT5 and not for SGLT1, and the enhancing effect of steroids on fructose uptake observed in rats fed SFA could be prevented by feeding PUFA (Table 23).

When adult rats are fed isocaloric diets varying in their type of lipids (SFA and PUFA), there is no difference in food intake or body weight gain [Thomson, 1982]. In contrast, when weaning rats are fed SFA or PUFA for four weeks, weight gain is approximately 30% higher in those fed PUFA as compared with SFA. This is not due to a difference in food intake, or to any alteration in the passive or carrier-mediated jejunal uptake of glucose or fructose. Indeed, ileal uptake of glucose was lower in PUFA than in SFA, and the jejunal as well as the ileal uptake of fructose was lower in PUFA than SFA. These studies do not provide an explanation for the greater weight gain in rats fed PUFA as compared with SFA. Clearly, however, the alterations in fructose uptake in animals given prednisone or budesonide could not be explained by differences in food intake or body weight gain. The enhanced villous height found in animals given budesonide and fed SFA as compared to PUFA could partially explain the effects on fructose uptake, at least for the animals given budesonide, but morphological alterations could not explain diet-associated alterations in those given control vehicle or prednisone. The lack of weight gain changes in animals given steroids was unexpected, but may be related to the relatively short duration of dosing (4 weeks).

In adult rats, feeding SFA compared with PUFA increases the value of the Vmax for glucose uptake in both the jejunum and ileum [Thomson et al., 1986]. In this study, the greater value of the ileal Vmax for glucose in rats fed SFA as compared with PUFA (Table 21) was associated with a decline rather than the expected increase in the abundance of SGLT1 (Table 26). Changes in either the Na^+/K^+-ATPase alpha 1 (the catalytic subunit) or the Na^+/K^+-ATPase beta 1 (the structural subunit) could not explain the alterations in glucose uptake, since the only variation observed was a reduction in abundance of Na^+/K^+-ATPase beta 1 in the jejunum of rats fed PUFA and given prednisone versus control vehicle (Table 25), and a reduction in ileal Na^+/K^+-ATPase alpha 1 mRNA expression in animals fed SFA and given budesonide (Table 26).

GLUT5 transports fructose across the BBM. The increased rates of uptake of fructose with budesonide or prednisone in rats fed SFA (Table 23), or the greater uptake in animals fed SFA versus PUFA (Table 24) were not parallelled by enhanced abundance of GLUT5 (Tables 29 and 30) or expression of GLUT5 mRNA expression (Tables 31 and 32). Dietary lipids modify the lipid composition and the physiochemical properties of the intestinal BBM [Keelan, 1996], and could potentially influence the transporter position in the membrane, and thereby alter the activity of the transporter. However, a change in the physiochemical properties of the BBM would be expected to alter the transporter activity of both SGLT1 and GLUT5, which did not occur.

GLUT2 transports glucose and fructose out of the enterocyte across the basolateral membrane. Recent studies suggest that GLUT2 may also be present in the BBM [Kellet and Heliwell, 2000;Heliwell et al., 2000a; Heliwell et al., 2000 b; Kellet, 2001]. We did not detect GLUT2 in the brush border membrane. GLUT2 abundance in the basolateral membrane did not explain the enhanced fructose uptake in animals fed SFA as compared to those fed PUFA, and the mRNA for GLUT2 did not correlate with protein expression such as in animals given prednisone and fed SFA or in animals given budesonide and fed SFA.

The immature enterocytes migrate from the crypt toward the villous tip, differentiating functionally as they progress [Keelan, 1996]. It is of interest that most glucose and fructose uptake occurs predominantly at the upper third of the villus [Wild et al., 1997]. In this study, we did not measure the uptake of sugars at different portions along the villus, but the abundances of SGLT1 and GLUT5 were similar in the upper as well as the lower portions of the villus (Table 33). A redistribution of the transporters from the base to the tip of the villus would be expected to be associated with greater sugar uptake, without necessarily any alteration on the total abundance of the transporters. Indeed, unlike the findings obtained with Western blotting, the administration of steroids or variations in the lipid content of the diet did influence the abundance of transporter along the jejunal villus when measured by immunohistochemistry. There was greater abundance of SGLT1 and GLUT5 in the jejunum of rats given control vehicle and fed SFA as compared with PUFA, and greater abundance of GLUT5 along the jejunal villus of rats fed either SFA or PUFA and given budesonide or prednisone as compared with control vehicle (Table 33). Thus, immunohistochemistry was more sensitive than Western blotting for demonstrating that the changes in transporter activity distribution was in fact associated with alterations in the abundance of SGLT1 or GLUT5. However, the uptake of fructose was not altered by steroids in rats fed PUFA (Table 23), even though the abundance of GLUT5 was increased (Table 33). Post-transcriptional modifications may be involved in this last situation. In other words, PUFA may prevent the effects observed with SFA on fructose uptake by acting at the post-transcriptional level. Another possibility would be that SFA acts on the enterocytes close to the crypts so that GLUT5 present in these cells would be able to transport fructose. PUFA may block or not act on this pathway, still requiring maturation of transporter in enterocytes along the crypt-villous unit in order to transport fructose.

Alterations in protein abundance and activity of transporters may be explained by post-translational regulation that occurs after the synthesis of the transporter protein. Events such as phosphorylation of transporter may explain differences between the glucose transporter abundance and their activity. Indeed, potential sites for protein kinase A and protein kinase C have been recognized in SGLT1, and the binding sites on SGLT1 for glucose are increased by phosphorylation [Hediger et al., 1987]. This could explain the discordance between the abundance of SGLT1 and the increased glucose uptake seen in this study when animals were fed SFA. These observations raise the possibility that the regulation of phosphorylation of SGLT1 leads to an alteration of its function, and could result in the control of glucose transport in the rat small intestine [Ishikawa et al., 1997]. It is possible that fatty acids or steroids influence the activity of protein C, and in doing so modulate the activity of growth factor receptors such as epidermal growth factor receptors [Johnson and McCormack, 1999]. In the intestine, several growth factors stimulate the migration of epithelial cells [Keelan, 1996]. It is possible that dietary lipids could affect cell migration by this pathway, and could also alter the expression of other molecules that can affect transporter activities once the transporter is in the membrane.

Steroids have been suggested to increase the expression of a series of transcription factors [Vanden Heuvel, 1999; Baxter, 1979; Bamberg et al., 1995; Thiele et al., 1999; Nosti-Escanilla et al., 1998]. Early response genes such as c-myc, c-jun and c-fos, have been demonstrated to be involved in processes of proliferation and differentiation, as well as ornithine decarboxylase (ODC), a key enzyme in the synthesis of polyamines and a requirement in any proliferative event [Bloom and Polak, 1982;

Sagor et al., 1983; Rountree et al., 1992]. Proglucagon has been shown to be involved the intestinal adaptive process [Mojsov et al., 1986; Orskov et al., 1987; Tappenden and McBurney, 1998]. For example, short chain fatty acids increase the ileal c-myc and proglucagon expression in rats undergoing intestinal resection [Tappenden and McBurney, 1998]. The enhanced ileal c-jun and proglucagon mRNA expression in animals fed SFA and given control vehicle suggests that dietary lipids such as SFA may signal the enhanced fructose uptake through proglucagon and c-jun. Potential new signals involved in the intestinal adaptive process have been identified by using of cDNA array technology [Stern et al., 2001; Erwin et al., 2000].

It was not established in this study what is the mechanism by which PUFA prevents the prednisone- or budesonide-associated enhanced uptake of fructose and lipids observed in rats fed SFA. It is evident that the abundance of SGLT1 and GLUT5 and their respective mRNAs do not clarify this mechanism. ERG and proglucagon may be involved in this intestinal adaptive response to steroids and to dietary lipids. This is true specifically in the ileum, where c-jun mRNA was increased in animals fed SFA as compared to those fed PUFA; and proglucagon mRNA was increased in animals given prednisone and fed PUFA. We did not measure the abundance of the ERGs and proglucagon, so we cannot discard possible involvement of these signals in this adaptive response.

In summary, 1) prednisone and budesonide when given in doses that are used clinically in humans enhance the intestinal uptake of fructose; 2) this enhancement may be increased further by feeding a diet enriched with saturated as compared with polyunsaturated fatty acids; 3) the adaptation is likely regulated by post-transcriptional as well as post-translational processes; and 4) this effect may be signalled by variations in the expression of early response genes and proglucagon.

Table 18: Body Weight Gain and Food Consumption

DIET	DRUG	FOOD INTAKE (g/day)	BODY WEIGHT GAIN (g/day)
Polyunsaturated	Control Vehicle	16.1 ± 0.4	8.8 ± 0.3
	Prednisone	16.9 ± 0.9	8.7 ± 0.3
	Budesonide	16.4 ± 0.4	8.7 ± 0.3
Saturated	Control Vehicle	17.5 ± 1.1	6.8 ± 0.6#
	Prednisone	16.3 ± 0.7	6.2 ± 0.5#
	Budesonide	16.6 ± 0.9	6.0 ± 0.4#

Mean ± SEM. There were eight animals in each group.

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA).

Table 19: Characteristics of Intestine

DIET	SITE	DRUG	WEIGHT (mg/cm)	% of INTESTINAL WALL COMPOSED OF MUCOSA	VILLOUS HEIGHT (µm)
Polyunsaturated	Jejunum	Control Vehicle	17.7 ± 1.8	52.2 ± 2.8	257 ± 31
		Prednisone	19.0 ± 2.5	44.1 ± 4.9	269 ± 15
		Budesonide	17.2 ± 1.5	54.4 ± 2.8	262 ± 16
	Ileum	Control Vehicle	11.9 ± 1.7	49.8 ± 3.8	270 ± 71
		Prednisone	12.5 ± 1.2	62.1 ± 6.2	314 ± 14
		Budesonide	13.6 ± 1.5	57.8 ± 6.8	181 ± 07~
Saturated	Jejunum	Control Vehicle	23.1 ± 1.9	49.3 ± 2.6	224 ± 37
		Prednisone	18.0 ± 1.0	48.7 ± 3.3	309 ± 23
		Budesonide	20.9 ± 1.5	56.8 ± 3.9	252 ± 17
	Ileum	Control Vehicle	11.4 ± 1.1	44.9 ± 4.4	211 ± 36
		Prednisone	9.3 ± 0.6	52.3 ± 5.6	261 ± 49
		Budesonide	10.4 ± 0.8	45.3 ± 2.5	239 ± 32#

Mean ± SEM

~, $p<0.05$, budesonide vs prednisone

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 20: Effects of Steroids on the intestinal uptake of D-glucose

DIET	SITE	DRUG	MAXIMAL TRANSPORT RATE Vmax (nmol.100 mg-1.min-1)	APPARENT MICHAELIS CONSTANT Km (mM)
Polyunsaturated	Jejunum	Control Vehicle	1454 ± 242	72 ±15
		Prednisone	1224 ± 151	54 ± 9
		Budesonide	1596 ± 250	62 ± 13
	Ileum	Control Vehicle	1233 ± 257	77 ± 20
		Prednisone	1352 ± 246	72 ± 17
		Budesonide	1090 ± 167	51 ± 11
Saturated	Jejunum	Control Vehicle	1285 ± 148	35 ± 6
		Prednisone	1696 ± 171	45 ± 6
		Budesonide	1461 ± 154	39 ± 6
	Ileum	Control Vehicle	2256 ± 423	100 ± 23
		Prednisone	3514 ± 838	185 ± 60
		Budesonide	2165 ± 355	93 ±19

Mean ± SEM

None of these differences were statistically significant.

Table 21: Effects of Dietary Lipids on the intestinal uptake of D-glucose

DRUG	SITE	DIET	MAXIMAL TRANSPORT RATE Vmax (nmol.100 mg-1.min-1)	APPARENT MICHAELIS CONSTANT Km (mM)
Control Vehicle	Jejunum	Polyunsaturated	1454 ± 242	72 ±15
		Saturated	1285 ± 148	35 ± 6
	Ileum	Polyunsaturated	1233 ± 257	77 ± 20
		Saturated	2256 ± 423#	100 ± 23
Prednisone	Jejunum	Polyunsaturated	1224 ± 151	54 ± 9
		Saturated	1696 ± 171	45 ± 6
	Ileum	Polyunsaturated	1352 ± 246	72 ± 17
		Saturated	3514 ± 838#	185 ± 60
Budesonide	Jejunum	Polyunsaturated	1596 ± 250	62 ± 13
		Saturated	1461 ± 154	39 ± 6
	Ileum	Polyunsaturated	1090 ± 167	51 ± 11
		Saturated	2165 ± 355#	93 ± 19

Mean ± SEM

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 22: Rates of intestinal uptake of L-glucose and D-mannitol

DIET	SITE		CONTROL VEHICLE	PREDNISONE	BUDESONIDE
Polyunsaturated	Jejunum				
		L-Glucose	10 ± 1	13 ± 1	10 ± 1
		D-Mannitol	12 ± 1	13 ± 1	10 ± 1
	Ileum				
		L-Glucose	12 ± 1	11 ± 1	8 ± 1
		D-Mannitol	11 ± 1	10 ± 1	10 ± 1
Saturated	Jejunum				
		L-Glucose	12 ± 1	13 ± 1	12 ± 1
		D-Mannitol	14 ± 1	15 ± 1	13 ± 1
	Ileum				
		L-Glucose	12 ± 1	11 ± 1	13 ± 1
		D-Mannitol	14 ± 1	13 ± 1	13 ± 1

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1}*min^{-1}*mM^{-1}

None of these differences was statistically significant.

Table 23: Effects of Steroids on intestinal uptake of D-fructose

DIET	DRUG	JEJUNUM	ILEUM
Polyunsaturated	Control Vehicle	12.4 ± 0.2	12.2 ± 0.5
	Prednisone	11.7 ± 0.4	10.8 ± 0.1
	Budesonide	12.7 ± 0.2	11.8 ± 0.6
Saturated	Control Vehicle	13.7 ± 0.1	17.3 ± 0.4
	Prednisone	16.8 ± 0.2*	16.5 ± 0.5
	Budesonide	15.3 ± 0.2*~	20.5 ± 1.0*~

Mean ± SEM of the slopes of the linear relationships between concentrations and rates of uptake of D-fructose.

The rates of uptake are expressed as nmol $*100$ mg tissue$^{-1}*$min^{-1}.

~, p<0.05, budesonide vs prednisone

*, p<0.05, budesonide or prednisone vs control vehicle

Table 24: Effect of Dietary Lipids on the intestinal uptake of D-fructose

DRUG	SITE	DIET	SLOPES
Control Vehicle	Jejunum	Polyunsaturated	12.4 ± 0.2
		Saturated	13.7 ± 0.#
	Ileum	Polyunsaturated	12.2 ± 0.5
		Saturated	17.3 ± 0.4#
Prednisone	Jejunum	Polyunsaturated	11.7 ± 0.4
		Saturated	16.8 ± 0.2#
	Ileum	Polyunsaturated	10.8 ± 0.1
		Saturated	16.5 ± 0.5#
Budesonide	Jejunum	Polyunsaturated	12.7 ± 0.2
		Saturated	15.3 ± 0.2#
	Ileum	Polyunsaturated	11.8 ± 0.6
		Saturated	20.5 ± 1.0#

Mean ± SEM

#, $p<0.05$; saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 25: Effect of Steroids on the protein abundance of SGLT1 and Na$^+$/K$^+$ -ATPase

DIET	SITE	DRUG	SGLT1	Na$^+$/K$^+$ -ATPase Alpha 1	Na$^+$/K$^+$ -ATPase Beta 1
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	1.07 ± 0.39	1.01 ± 0.43	0.82 ± 0.09*
		Budesonide	0.99 ± 0.27	1.20 ± 0.43	0.94 ± 0.20
	Ileum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	0.64 ± 0.15*	1.01 ± 0.36	0.87 ± 0.48
		Budesonide	0.83 ± 0.07*~	0.96 ± 0.11	1.02 ± 0.26
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	1.38 ± 1.15	0.98 ± 0.25	0.94 ± 0.25
		Budesonide	1.06 ± 0.50	0.78 ± 0.40	0.79 ± 0.26
	Ileum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	0.88± 0.77	1.20 ± 0.35	0.77 ± 0.46
		Budesonide	0.56 ± 0.1[illegible]	1.92 ± 1.39	0.99 ± 0.22

Mean ± SD

~, $p<0.05$, budesonide vs prednisone

*, $p<0.05$, budesonide or prednisone vs control vehicle

Table 26: Effect of Dietary Lipids on the protein abundance of SGLT1 and Na^+/K^+ - ATPase

DRUG	SITE	DIET	SGLT1	Na^+/K^+ -ATPase Alpha 1	Na^+/K^+ - ATPase Beta 1
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.02 ± 0.97	1.01 ± 0.23	1.52 ± 0.85
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.71 ± 0.43	0.77 ± 0.63	1.421 ± 0.86
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.84 ± 1.01	0.84 ± 0.26	1.21 ± 0.28
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.38 ± 0.46	0.56 ± 0.68	1.56 ± 1.71
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.53 ± 0.40#	0.72 ± 0.38	1.09 ± 0.69
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.49 ± 0.12#	0.68 ± 0.32#	0.90 ± 0.35

Mean ± SD

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 27: Effect of Steroids on the mRNA Expression of SGLT1 and Na^+/K^+-ATPase

DIET	SITE	DRUG	SGLT1	Na^+/K^+-ATPase Alpha 1	Na^+/K^+-ATPase Beta 1
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	1.37 ± 0.29*	2.40 ± 3.46	1.99 ± 1.05
		Budesonide	1.02 ± 0.66	1.75 ± 0.85	2.08 ± 0.82
	Ileum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	1.52 ± 0.69	1.83 ± 1.49	0.96 ± 0.76
		Budesonide	1.18 ± 0.51	2.91 ± 2.40	1.46 ± 0.70
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	0.99 ± 0.31	0.92 ± 0.16	1.04 ± 0.30
		Budesonide	1.00 ± 0.26	0.83 ± 0.30	0.84 ± 0.38
	Ileum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
		Prednisone	0.82 ± 0.50	1.00 ± 1.06	2.07 ± 2.54
		Budesonide	0.94 ± 0.28	1.11 ± 0.92	0.88 ± 0.58

Mean ± SD

~, $p<0.05$, budesonide vs prednisone

*, $p<0.05$, budesonide or prednisone vs control vehicle

Table 28: Effect of Dietary Lipids on the mRNA expression of SGLT1 and Na$^+$/K$^+$-ATPase

DRUG	SITE	DIET	SGLT1	Na$^+$/K$^+$ -ATPase Alpha 1	Na$^+$/K$^+$ - ATPase Beta 1
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.27 ± 0.75	1.46 ± 0.62	1.79 ± 0.69#
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.37 ± 0.89	1.86 ± 1.53	1.85 ± 1.26
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.98 ± 0.19	0.56 ± 0.48#	1.05 ± 0.53
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.73 ± 0.26#	0.85 ± 0.15#	1.57 ± 0.60
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.89 ± 0.08#	0.71 ± 0.12	0.72 ± 0.37
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.77 ± 0.31#	1.57 ± 1.79	0.85 ± 0.30

Mean ± SD

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 29: Effect of Steroids on the protein abundance of GLUT5 and GLUT2

DIET	SITE	DRUG	GLUT5	GLUT2
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.28 ± 0.63	1.09 ± 0.58
		Budesonide	1.36 ± 0.63	1.17 ± 0.26
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	0.90 ± 0.19	1.00 ± 0.33
		Budesonide	1.04 ± 0.09	1.17 ± 0.36
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.36 ± 0.33*	1.03 ± 0.52
		Budesonide	0.84 ± 0.23~	1.08 ± 0.48
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	0.66 ± 0.28*	1.20 ± 0.52
		Budesonide	0.86 ± 0.15	1.07 ± 0.36

Mean ± SD

~, $p<0.05$, budesonide vs prednisone

*, $p<0.05$, budesonide or prednisone vs control vehicle

Table 30: Effect of Dietary Lipids on the protein expression of GLUT5 and GLUT2

DRUG	SITE	DIET	GLUT5	GLUT2
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.87 ± 0.60	1.25 ± 0.51
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	1.92 ± 3.31	0.92 ± 0.34
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.70 ± 0.48	1.86 ± 1.77
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.40 ± 0.26	1.56 ± 1.21
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.54 ± 0.31	1.05 ± 0.36
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.52 ± 0.20	1.05 ± 0.66

Mean ± SD

None of these differences was statistically significant.

Table 31: Effect of Steroids on the mRNA expression of GLUT5 and GLUT2

DIET	SITE	DRUG	GLUT5	GLUT2
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.48 ± 0.57	1.41 ± 0.31
		Budesonide	1.04 ± 0.19	1.14 ± 0.31
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	5.47 ± 7.60	0.96 ± 0.42
		Budesonide	7.16 ± 9.99	1.03 ± 0.46
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.08 ± 0.10	1.01 ± 0.26
		Budesonide	0.93 ± 0.35	1.23 ± 0.65
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.04 ± 1.17	1.62 ± 0.93
		Budesonide	1.17 ± 0.35	1.10 ± 0.27

Mean ± SD

None of these differences was statistically significant.

Table 32: Effect of Dietary Lipids on the mRNA expression of GLUT5 and GLUT2

DRUG	SITE	DIET	GLUT5	GLUT2
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.87 ± 0.60	1.00 ± 0.70
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	1.92 ± 3.31	1.53 ± 0.62
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.70 ± 0.48	0.72 ± 0.20#
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.40 ± 0.26	0.86 ± 0.22
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.54 ± 0.31	0.64 ± 0.13#
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.52 ± 0.20	0.96 ± 0.49

Mean ± SD

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 33: Effect of dietary lipids and steroids on the immunohistochemistry of SGLT1 and GLUT5 along the jejunal villus

SGLT1	Saturated Fatty Acid Diet			Polyunsaturated Fatty Acid Diet		
Villus Section	Control Vehicle	Budesonide	Prednisone	Control Vehicle	Budesonide	Prednisone
1(tip)	5.77 ± 0.72#	11.98 ± 4.81	8.27 ± 4.25	3.98 ± 0.72	11.24 ± 3.28	5.83 ± 1.23
2	6.70 ± 1.53	10.39 ± 3.24	8.29 ± 2.89	5.73 ± 1.34	11.35 ± 4.54	7.12 ± 2.04
3	7.89 ± 1.55#	9.97 ± 1.96	10.45 ± 3.03	5.40 ± 1.25	13.23 ± 5.52	9.04 ± 2.68
4	8.00 ± 0.86#	8.63 ± 1.32	11.47 ± 4.65	5.81 ± 1.14	12.28 ± 4.93	7.97 ± 2.33
5(crypt)	6.83 ± 0.64#	6.15 ± 0.49	6.71 ± 2.21	4.48 ± 094	8.45 ± 3.32	5.65 ± 1.08
total	35.50 ± 7.87	51.92±18.01	41.33±26.20	24.55 ± 7.43	63.79 ±35.89	38.31± 14.30

GLUT5	Saturated Fatty Acid Diet			Polyunsaturated Fatty Acid Diet		
Villus Section	Control Vehicle	Budesonide	Prednisone	Control Vehicle	Budesonide	Prednisone
1(tip)	3.45 ± 0.44	10.51±4.47*	8.88±0.51*#	3.61 ± 0.98	12.08± 3.30*	11.78± 1.18*
2	3.94 ± 1.01	10.56±4.52*	7.59 ± 1.95	3.67 ± 1.00	9.73± 1.91*	9.33± 0.99*
3	4.98 ± 0.70#	12.29±2.91*	10.31±3.21*	3.47 ± 0.86	11.89± 2.82*	10.45± 1.12*
4	5.41 ± 0.78#	10.97±2.31*	7.66 ± 2.27	3.53 ± 0.41	10.62± 1.55*	8.69± 1.08*
5(crypt)	3.41 ± 0.63	6.34 ± 1.29	5.29 ± 0.93#	2.56 ± 0.37	8.20± 1.89*	7.16± 0.98*
total	20.27 ± 3.55	42.52±16.52	41.35±8.90	15.96 ± 5.56	48.60±18.54	48.57± 6.85

Density *area

mean ± SD

*, p<0.05, budesonide or prednisone vs control

#, p<0.05, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 34: Effect of Steroids on the mRNA Expression of Early Response Genes

DIET	SITE	DRUG	c-jun	c-myc	c-fos
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	n.s.
		Prednisone	1.04 ± 0.85	0.92 ± 0.33	n.s.
		Budesonide	0.85 ± 0.10	0.76 ± 0.24	n.s.
	Ileum	Control Vehicle	1 ± 0	1 ± 0	n.s.
		Prednisone	1.16 ± 0.37	1.09 ± 0.05	n.s.
		Budesonide	1.74 ± 0.92	0.86 ± 0.49	n.s.
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	n.s.
		Prednisone	1.07 ± 0.15	1.02 ± 0.18	n.s.
		Budesonide	1.02 ± 0.13	1.03 ± 0.10	n.s.
	Ileum	Control Vehicle	1 ± 0	1 ± 0	n.s.
		Prednisone	0.67 ± 0.26	0.98 ± 0.11	n.s.
		Budesonide	0.73 ± 0.44	1.08 ± 0.22	n.s.

Mean ± SD

n.s. = no signal

None of these differences was statistically significant.

Table 35: Effect of Dietary Lipids on the mRNA Expression of Early Response Genes

DRUG	SITE	DIET	c-jun	c-myc	c-fos
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	n.s
		Saturated	0.80 ± 0.26	0.90 ± 0.25	n.s
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	n.s
		Saturated	2.14 ± 0.79#	0.91 ± 0.58	n.s
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	n.s
		Saturated	0.93 ± 0.50	0.85 ± 0.19	n.s
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	n.s
		Saturated	1.32 ± 0.83	0.78 ± 0.45	n.s
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	n.s
		Saturated	0.94 ± 0.19	1.02 ± 0.50	n.s
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	n.s
		Saturated	0.85 ± 0.36	1.11 ± 0.26	n.s

Mean ± SD

n.s. = no signal

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

Table 36: Effect of Steroids on the mRNA expression of proglucagon and ODC

DIET	SITE	DRUG	Proglucagon	ODC
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	4.30 ± 4.89	1.20 ± 0.96
		Budesonide	1.02 ± 0.22	0.89 ± 0.16
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.51 ± 0.13*	1.43 ± 0.32
		Budesonide	1.12 ± 0.13~	1.08 ± 0.48
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.15 ± 0.22	0.89 ± 0.44
		Budesonide	1.03 ± 0.02*	0.92 ± 0.52
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	0.71 ± 0.22	0.90 ± 0.14
		Budesonide	0.41 ± 0.06	0.82 ± 0.49

Mean ± SD

~, $p<0.05$, budesonide vs prednisone

*, $p<0.05$, budesonide or prednisone vs control vehicle

Table 37: Effect of Dietary Lipids on the mRNA expression of proglucagon and Ornithine Decarboxylase (ODC)

DRUG	SITE	DIET	Proglucagon	ODC
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.44 ± 0.49	0.97 ± 0.25
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	2.15 ± 0.35#	1.14 ± 0.74
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.12 ± 0.01#	1.20 ± 0.69
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.99 ± 0.24	0.91 ± 0.44
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.40 ± 0.42	0.99 ± 0.57
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.78 ± 0.10	0.77 ± 0.23

Mean ± SD

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

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I) THE STIMULATING EFFECT OF STEROIDS ON INTESTINAL LIPID UPTAKE IN RATS IS ENHANCED BY FEEDING A SATURATED FATTY ACID DIET

1) Introduction

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis [Brattsand, 1990; Brignola et al., 1992; Brignola et al., 1994; Danielson and Prytz, 1994; Lofberg et al., 1993; Thiesen and Thomson; 1996]. However, systemic steroids may be associated with numerous and potentially serious adverse effects [Brattsand, 1990; Ehrich et al., 1992; Girdwood and Petrie, 1987; Greenberg, 1994; Haynes and Murad, 1985]. For this reason, non-systemic steroids have been developed. The non-systemic steroid budesonide has high topical activity, low systemic bioavailability, as well as rapid first pass metabolism in the liver and intestine [Brattsand, 1990]. Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease [Danielsson et al., 1992].

Intestinal adaptation is a process that occurs in response to physiological or pathological processes such as intestinal resection, aging, diabetes, abdominal radiation, chronic alcohol intake, and feeding diets of varying lipid, protein or carbohydrate composition [Thomson et al., 1990; Thomson and Wild, 1997a; Thomson and Wild, 1997b]. Thus, there are different morphological and physiological characteristics of the intestine depending upon the nutrient composition of the diet [Diamond and Karasov, 1984; Thomson and Rajotte, 1983a; Thomson and Rajotte, 1983b; Thomson, 1982]. In adult rats, the intestinal uptake of sugars and lipids is enhanced by feeding an isocaloric semisynthetic diet enriched with saturated as compared with polyunsaturated fatty acids [Thomson et al., 1986].

In young animals, steroids induce precocious development of the intestinal brush border membrane (BBM) enzymes, and facilitate the induction of several BBM enzymes by dietary carbohydrate [Batt and Peters, 1976]. Steroids given by mouth enhance intestinal glucose absorption by adult animals [Batt and Peters, 1975; Batt and Scott, 1982]. Dexamethasone given subcutaneously blunts the expected adaptive response following intestinal resection [Park et al., 1994].

Proglucagon-derived peptides originate from the processing and breakage of the proglucagon gene product [Mojsov et al., 1986; Orskov et al., 1987] in the L-cells present in the ileum and colon [Larsson et al., 1975]. It has been suggested that the mRNA levels of proglucagon and ornithine decarboxylase (ODC), as well as of early response genes (ERGs) such as c-myc, c-jun and c-fos, are involved in the adaptive process of the intestine such as small intestinal resection [Bloom and Polak, 1982; Rountree et al., 1992; Sagor et al., 1983; Stern et al., 2001]. ODC, a key enzyme in the synthesis of polyamines and a requirement in any proliferative event, may increase the transport of nutrients inducing proliferation and differentiation of enterocytes and consequently thereby increase the number of transporters. It is unknown if proglucagon, ODC or ERGs are involved in the process of intestinal adaptation to steroids or to changes in dietary lipids.

A wide variety of cytokines are produced locally by the intestinal epithelium, and they may be essential for the homeostasis of the intestinal tissue during development [Fiochi, 1997; Kagnoff et al., 1996]. Cytokine gene expression has been shown to be regulated by hydrocortisone and by dexamethasone during postnatal small intestinal development (34). Administration of IL1α, IL-6, and IL-8 increase the *in vitro* uptake of glucose (19). It is not known if the cytokines in the intestine are influenced by steroids or by dietary lipids.

The intestinal absorption of lipids is thought to involve a process of passive diffusion, but several lipid binding proteins in the BBM or in the cytosol of the enterocyte may be involved in lipid uptake [Besnard and Niot, 1999; Clandinin and Thomson, 1999; Reshef et al., 1992; Schoeller et al., 1995]. The role of these lipid binding proteins in the adaptation of lipid uptake is unclear. The effect of locally or systemically acting steroids on intestinal lipid transport in animals fed diets enriched with saturated or polyunsaturated fatty acids has not been described. We formulated the hypothesis that feeding weanling rats a saturated as compared with a polyunsaturated diet will enhance the increased intestinal uptake of lipids observed when animals are given steroids. The results show that 1) prednisone and budesonide given in doses used clinically in humans increase the uptake of several long-chain fatty acids and cholesterol; 2) this increase can be enhanced by feeding a diet enriched with saturated as compared with polyunsaturated fatty acids; 3) this adaptation of lipid uptake does not appear to involve changes in the mRNA expression of the liver or the ileal lipid binding proteins in the enterocyte; and 4) dietary lipid-signalling of this adaptive response may involve c-jun, proglucagon, TNF-α and IL-10, whereas steroid-signalling may involve proglucagon.

2) Methods and Materials

2.1) Northern Immunoblotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E.coli*) were transformed with plasmids containing the desired DNA sequences to be used as a probe for the Northern blotting. ERG probes were obtained from Oncogene Research Products; cDNA probe encoding proglucagon was obtained from Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; ODC probe was obtained from Dr. Blackshear, University of Chicago; L-FABP and ILBP probes were provided by Dr. Agellon, University of Alberta; and TNF-α, IL-2, IL-6 and IL-10 were obtained from BIO/CAN Scientific. A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labelled DNA (Roche Diagnostics, Quebec, CA).

RNA was extracted from the jejunum and ileum of at least three animal in each group. These segments were homogenized in a denaturing solution, containing guanidinium thiocyanate, using the biorad fast prep shaking centrifuge. Following addition of 2 M sodium acetate, a phenol chloroform extraction was done. The upper aqueous phase was transferred to a tube and the RNA was precipitated with isopropanol and washed with 70% ethanol. RNA samples were stored at -70°C.

Total RNA was separated based on molecular weight as it was electrophoresed through a denaturing agarose gel (1.16% agarose). The migration lasted 5 hours at a current level of 100 volts in an electrophoresis tank (HLB12 Complete Horizontal Long Bed Gel System, Tyler, Edmonton, CA). RNA was then transferred from the gel to a nylon membrane by capillary action overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA onto the membranes. As a pre-hybridization, membranes were incubated during 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at 42°C overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid) in order to reduce non-specific binding sites. They were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was done using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA) and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA). To determine the exact RNA quantity that has been loaded, the 28S ribosomal band density on the membrane was evaluated after the transfer.

3) RESULTS

3.1) Animal Characteristics

Giving prednisone and budesonide had no effect on food intake or body weight gain (Table 18). In the control vehicle, prednisone and budesonide groups, the weight gain was higher in rats fed PUFA as compared with SFA. Prednisone and budesonide had no effect on the total weight of the intestine, or on the percent of the intestinal wall comprised of mucosa (Table 19). Also, steroids had no effect on the villous height of the jejunum or ileum of rats fed SFA, or for the jejunum of animals fed PUFA. In the ileum of rats fed PUFA, the villous height was higher ($p<0.05$) in animals given prednisone as compared with budesonide. In the ileum of rats given budesonide, the height of the villi was less in those fed PUFA than SFA (Table 19).

3.2) Lipid Uptake

In rats fed PUFA, prednisone increased the jejunal uptake of 12:0, 18:1 and 18:2, and increased the ileal uptake of 18:3 (Table 38). In rats fed SFA and given prednisone, there was no change in the jejunal uptake of lipids, whereas the rates of ileal uptake of 12:0 and 18:2 were increased and the uptake of 16:0 was decreased. Giving budesonide to rats fed PUFA increased the jejunal uptake of 16:0, 18:1 and 18:2, and enhanced the ileal uptake of 18:1. In rats fed PUFA, the rates of jejunal uptake of 16:0 and the ileal uptake of 18:1 were higher with budesonide than with prednisone. In those animals fed SFA, the rates of ileal uptake of 12:0, 18:0 and 18:2 were lower with budesonide than with prednisone. In rats fed SFA, budesonide increased the jejunal uptake of 12:0, decreased the jejunal uptake of cholesterol, and reduced the ileal uptake of 16:0.

In rats given prednisone or budesonide, the uptake of many fatty acids was higher when the animals were fed SFA rather than PUFA. For example, in rats given prednisone the rates of jejunal uptake of fatty acids 12:0, 16:0, 18:0, 18:1 and 18:3 as well as cholesterol were approximately 50% greater in those fed SFA as compared with PUFA.

3.3) mRNA Expression of L-FABP and ILBP

In rats fed PUFA, giving budesonide reduced the jejunal mRNA expression of L-FABP, and increased ileal expression of ILBP, as compared with control vehicle (Table

39). In rats fed SFA, budesonide reduced the ileal mRNA expression of ILBP and prednisone increased the mRNA expression of L-FABP (Table 40).

3.4) Early Response Gene (ERG) mRNA Expression

Steroids had no effect on the expression of the mRNAs for ERGs in animals feed SFA or PUFA (Table 34). The c-jun expression was increased in the ileum of animals given control vehicle and fed SFA as compared to PUFA (Table 35), but otherwise dietary lipids had no effect on the expression of the mRNAs for ERGs.

3.5) Proglucagon and ODC mRNA expression

Neither steroids nor dietary lipids altered the ODC mRNA expression (Tables 36 and 37). Prednisone increased the ileal proglucagon mRNA expression in animals fed PUFA (Tables 36). In rats given control vehicle, SFA increased the ileal expression of proglucagon mRNA as compared with PUFA. In animals given prednisone, SFA decreased the jejunal expression of proglucagon mRNA as compared with those fed PUFA (Table 37).

3.6) Cytokine mRNA Expression

Giving steroids had no effect on the mRNA expression of TNF-α, IL-2, IL-6 or IL-10 (Table 41). Dietary lipids did not change the mRNA expression of IL-2 or IL-6, but the ileal expression of mRNA for TNF-α was reduced in animals fed PUFA and given control vehicle. Animals given budesonide and fed PUFA also had a reduction in the expression of mRNA for IL-10 in both jejunum and ileum (Table 42).

4) Discussion

When adult rats are fed isocaloric semisynthetic diets varying in the type of lipids (SFA or PUFA), there is no difference in food intake or body weight gain [Thomson et al., 1986]. In contrast, when weanling rats are fed SFA or PUFA for four weeks, weight gain is approximately 30% higher in those fed PUFA as compared with SFA (Table 18). This is not due to a difference in food intake, or to any alteration in the passive or carrier-mediated jejunal uptake of glucose or fructose (Tables 18, 21 and 24). The differences in weight gain are also not explained by changes in lipid uptake (Table 38). These studies do not provide an explanation for the greater weight gain in rats fed PUFA than SFA.

The effect of budesonide on lipid uptake depends upon the lipid composition of the diet. In animals fed PUFA, budesonide increases the rate of jejunal uptake of the long-chain length fatty acids 16:0, 18:1 and 18:2 as well as the ileal uptake of 18:1 (Table 38). In animals fed SFA, giving budesonide reduces the jejunal uptake of cholesterol and the ileal uptake of 16:0. By the same token, giving prednisone to animals fed PUFA increases the jejunal uptake of 12:0, 18:1 and 18:2, and as well increases the ileal uptake of 18:3. Giving prednisone to rats fed SFA has no effect on lipid uptake in the jejunum, reduces ileal uptake of 16:0, and increases ileal uptake of 12:0 and 18:2. However, when comparing lipid uptake in rats given prednisone or budesonide, uptake was usually lower in those fed PUFA than SFA. Thus, the ability of steroids to alter intestinal lipid uptake depends upon the lipid content of the diet, and feeding PUFA generally diminishes the increase in lipid uptake observed in rats fed SFA. These alterations in lipid uptake in animals given prednisone or budesonide could not be explained by differences in food intake, body weight gain, or villous height (Tables 18 and 19).

Intestinal lipid uptake occurs by a process of passive permeation [Clanidinin and Thomson, 1999; Perin et al., 1997], but a component of the uptake of long-chain fatty acids is mediated by the sodium/hydrogen exchanger [Poirier, 1996] and/or by fatty acid binding proteins in the brush border membrane or in the cytosol of the enterocyte [Besnard and Niot, 199; Poirier, 1996; Schoeller et al., 1995]. The increased jejunal and ileal uptake of lipids in rats given prednisone or budesonide (Table 38) does not distinguish between which of these passive or protein-mediated steps may be affected by giving steroids. Adaptations in lipid uptake due to changes in the fat content of the diet are associated with alterations in the phospholipid content of the BBM [Thomson et al., 1986]. However, any steroid-associated alteration in the lipophilic properties of the BBM would be expected to change the uptake of all lipids, and this did not occur (Table 38).

The role of the lipid binding proteins in the enterocyte in the overall control of lipid absorption remains to be determined [Besnard and Niot, 1999]. In this study, there were steroid- and dietary lipid-associated alterations in lipid uptake in the jejunum and ileum (Table 38), without parallel changes in the expression of the mRNAs for either L-FABP or ILBP (Tables 39 and 40). We did not assess the effect of steroids and dietary lipids on the protein abundance of these lipid binding proteins, so it is possible that these proteins do indeed play a role in the adaptation of lipid uptake, but that their abundance is controlled by post-transcriptional processes. Also, we did not assess the expression of the mRNAs for fatty acid transporters in the BBM or for I-FABP in the cytosol, so we do not know if a change in some other protein-mediated component is important in the diet- or steroid-associated alterations in lipid uptake.

The variations in the expression of the mRNA for c-jun, proglucagon, TNF-α and IL-10 mRNA expression in animals fed SFA (Tables 42) suggests that dietary lipids such as SFA may signal the enhanced lipid uptake through these proteins. These findings lead us to propose a model by which SFA would alter the uptake of long chain fatty acids and cholesterol: initially, the modification in the diet would be sensed at the level of the intestinal epithelium, in which the lipid is absorbed and alters the gene expression of c-jun, followed by up-regulation of proglucagon and down-regulation of certain cytokines which would be responsible for the phenotypic changes in lipid transport. It is intriguing that the dietary lipid effect on the cytokines involved an inflammatory cytokine, TNF-α, and an anti-inflammatory cytokine, IL-10. On the other hand, prednisone increased the ileal expression of proglucagon in animals fed PUFA, as well as budesonide in those fed SFA (Table 36). Thus, the signals for adaptation of lipid uptake differ between dietary lipids and between steroids. Possible new signals involved in this intestinal adaptive response may be involved, such as the ones recently identified in the resection model by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001].

We did not establish the mechanism by which PUFA prevents the prednisone- or budesonide-associated enhancement in the uptake of lipids observed in rats fed SFA. Clearly, these locally and systemically active steroids, when given in doses which are effective clinically in humans, modify the intestinal absorption of lipids by a process which is influenced by the dietary content of lipids. Dietary lipid signalling of this adaptive response may involve c-jun, proglucagon, TNF-α and IL-10, whereas steroid signalling may involve proglucagon. The clinical significance of these observations needs to be established. We speculate that the administration of a polyunsaturated diet may be useful to reduce the potential for the enhanced absorption of lipids which may occur in persons treated with steroids.

Table 38: Rates of Intestinal Uptake of Fatty Acids and Cholesterol

DIET	SITE	CONTROL VEHICLE	PREDNISONE	BUDESONIDE
Polyunsaturated	Jejunum			
	12:0	49.6 ± 0.23	65.7 ± 4.2 *	59.7 ± 3.8
	16:0	11.8 ± 1.1	11.3 ± 1.0	15.4 ± 1.0 ~*
	18:0	10.9 ± 0.8	11.6 ± 0.8	12.6 ± 0.8
	18:1	8.8 ± 0.8	12.2 ± 1.0 *	13.0 ± 0.9 *
	18:2	8.4 ± 0.7	13.3 ± 1.0 *	14.4 ± 1.2 *
	18:3	5.5 ± 0.5	5.6 ± 0.4	6.8 ± 0.4
	Cholesterol	16.0 ± 1.8	13.2 ± 1.6	8.6 ± 0.08
	Ileum			
	12:0	50.9 ± 0.27	52.6 ± 2.9	59.2 ± 3.3
	16:0	12.6 ± 1.2	13.2 ± 1.3	16.1 ± 1.2
	18:0	14.2 ± 2.2	11.7 ± 1.0	11.3 ± 0.6
	18:1	8.8 ± 0.9	8.2 ± 0.8	12.3 ± 0.8 ~*
	18:2	8.4 ± 0.5	11.1 ± 0.8	11.1 ± 0.9
	18:3	4.6 ± 0.5	0.63 ± 0.4 *	5.7 ± 0.3
	Cholesterol	17.0 ± 2.0	14.8 ± 1.4	13.0 ± 1.0
Saturated	Jejunum			
	12:0	67.1 ± 4.4 #	78.3 ± 3.7 #	84.1 ± 4.3 #*
	16:0	17.2 ± 1.1 #	16.7 ± 1.1 #	15.7 ± 1.4
	18:0	17.1 ± 2.0 #	18.3 ± 1.2 #	15.9 ± 1.3 #
	18:1	14.5 ± 1.0 #	17.1 ± 1.2 #	14.2 ± 1.7
	18:2	19.1 ± 1.7 #	15.7 ± 1.4	14.6 ± 1.6
	18:3	9.4 ± 0.7 #	8.2 ± 0.6 #	7.6 ± 0.8
	Cholesterol	28.6 ± 3.4 #	22.0 ± 2.4 #	17.6 ± 1.6 *
	Ileum			
	12:0	66.8 ± 8.0	128.8 ± 6.8 # *	82.9 ± 5.6#~
	16:0	18.9 ± 1.7	13.8 ± 1.2 *	13.0 ± 0.9 *
	18:0	15.7 ± 1.4	17.8 ± 1.8	12.0 ± 1.1 # ~
	18:1	13.9 ± 1.7 #	15.0 ± 1.4	11.4 ± 1.1 #
	18:2	12.0 ± 1.4 #	15.9 ± 1.5 *	10.2 ± 0.8 # ~
	18:3	7.4 ± 0.9 #	9.3 ± 1.1	6.8 ± 0.6 #
	Cholesterol	25.6 ± 2.8 #	22.0 ± 1.8#	18.0 ± 1.8 #

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1}*min^{-1}*mM^{-1}

~, p<0.05, budesonide vs prednisone

#, p<0.05, polyunsaturated (PUFA) vs saturated diet (SFA)

*, p<0.05, budesonide or prednisone vs control vehicle

Table 39: Effect of steroids on the mRNA Expression of L-FABP and ILBP

DIET	SITE	DRUG	L-FABP	ILBP
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	n.s.
		Prednisone	0.82 ± 0.10	n.s.
		Budesonide	0.54 ± 0.35*	n.s.
	Ileum			
		Control Vehicle	1 ± 0	1 ± 0
		Prednisone	0.93 ± 0.35	1.11 ± 0.23
		Budesonide	1.18 ± 0.32	1.53 ± 0.17 #*
Saturated	Jejunum	Control Vehicle	1 ± 0	n.s.
		Prednisone	1.07 ± 0.18	n.s.
		Budesonide	1.21 ± 0.59	n.s.
	Ileum	Control Vehicle	1 ± 0	1 ± 0
		Prednisone	1.69 ± 1.89	1.72 ± 1.83
		Budesonide	0.78 ± 0.23	0.56 ± 0.40

Mean ± SD.

L-FABP, liver fatty acid binding protein

ILBP, ileal binding protein

n.s., no signal

*, $p<0.05$, budesonide or prednisone vs control vehicle

#, $p<0.05$, budesonide or prednisone.

Table 40: Effect of dietary lipids on the mRNA expression of L-FABP and ILBP

DRUG	SITE	DIET	L-FABP	ILBP
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	n.s.
		Saturated	0.89 ± 0.31	n.s.
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	1.17 ± 0.74	1.65 ± 1.28
Prednisone	Jejunum	Polyunsaturated	1 ± 0	n.s.
		Saturated	1.15 ± 0.36	n.s.
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	1.57 ± 0.34 #	1.19 ± 0.15
Budesonide	Jejunum	Polyunsaturated	1 ± 0	n.s.
		Saturated	1.47 ± 0.63	n.s.
	Ileum	Polyunsaturated	1 ± 0	1 ± 0
		Saturated	0.78 ± 0.57	0.40 ± 0.19 #

Mean ± SD

L-FABP, liver fatty acid binding protein

ILBP, ileal binding protein

n.s., no signal

#, $p<0.05$, saturated diet (SFA) vs polyunsaturated (PUFA)

Table 41: Effect of steroids on the cytokine gene expression

DIET	SITE	DRUG	TNF-α	IL-2	IL-6	IL-10
Polyunsaturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Prednisone	0.84 ± 0.11	1.06 ± 0.14	0.83 ± 0.12	0.98 ± 0.02
		Budesonide	0.92 ± 0.19	1.08 ± 0.06	0.87 ± 0.14	0.99 ± 0.14
	Ileum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Prednisone	0.86 ± 0.08	0.89 ± 0.34	0.91 ± 0.02	1.18 ± 0.12
		Budesonide	0.67 ± 0.33	0.92 ± 0.24	0.71 ± 0.39	1.20 ± 0.22
Saturated	Jejunum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Prednisone	1.07 ± 0.33	1.03 ± 0.38	1.17 ± 0.29	1.01 ± 0.09
		Budesonide	0.91 ± 0.15	0.99 ± 0.29	0.99 ± 0.17	0.71 ± 0.32
	Ileum	Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Prednisone	1.59 ± 0.68	1.52 ± 1.06	1.05 ± 0.26	0.83 ± 0.43
		Budesonide	1.03 ± 0.27	0.77 ± 0.40	1.02 ± 0.41	0.56 ± 0.39

Mean ± SD

None of these differences was statistically significant.

n.s., no signal

Table 42: Effect of dietary lipids on cytokine gene expression

DRUG	SITE	DIET	TNF-α	IL-2	IL-6	IL-10
Control Vehicle	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.79 ± 0.25	1.02 ± 0.29	0.76 ± 0.26	0.81 ± 0.33
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.50 ± 0.24 #	1.13 ± 1.03	0.78 ± 0.55	1.81 ± 0.89
Prednisone	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.97 ± 0.19	0.67 ± 0.42	1.02 ± 0.04	0.54 ± 0.36
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.95 ± 0.55	1.32 ± 0.45	0.82 ± 0.46	1.06 ± 0.04
Budesonide	Jejunum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.80 ± 0.26	0.90± 0.29	0.87 ± 0.25	0.52 ± 0.17 #
	Ileum	Polyunsaturated	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.88 ± 0.46	0.68 ± 0.24	0.99 ± 0.21	0.69 ± 0.19 #

Mean ± SD

#, $p<0.05$, saturated fatty acid diet (SFA) versus polyunsaturated (PUFA)

5) References

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J) THE LOCALLY ACTING GLUCOCORTICOSTEROID BUDESONIDE ENHANCES INTESTINAL SUGAR UPTAKE FOLLOWING INTESTINAL RESECTION IN RATS

1) Introduction

The topic of intestinal adaptation has been reviewed [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, there is hyperplasia of the remaining intestine, which may be accompanied by enhanced uptake of nutrients [Dowling and Booth, 1967; Weiser, 1973; Kinter and Wilson, 1975; Garvey et al., 1976; Hanson et al., 1977; King et al., 1981]. The signals which mediate this adaptive process may include proglucagon-derived peptides, ornithine decarboxylase (ODC), and early response genes (ERGs)[Bristol and Williamson, 1988; Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998]. Proglucagon-derived peptides originate from processing and breakage of the proglucagon gene [Mojsov et al., 1986; Orskov et al., 1987] in the L-cells present in the ileum and colon [Larsson et al., 1975]. The mRNA levels of proglucagon, ODC, as well as ERGs such as c-myc, c-jun and c-fos have been suggested to be involved in the adaptive process of the remaining intestine after jejunoileal resection [Bristol and Williamson, 1988; Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998]. It is unknown if proglucagon, ODC or ERGs in the intestine are influenced by steroids. Other possible signals have been recently identified by cDNA microarray analysis and may have a role in this intestinal adaptive model [Erwin et al., 2000; Stern et al., 2001].

The Na^+ -gradient across the brush border membrane (BBM) provides the driving force for glucose transport in the enterocyte [Ricklis and Quastel, 1958]. This gradient is maintained by the action of the Na^+/K^+-ATPase which is restricted to the basolateral membrane (BLM)[Hirayama et al., 1992]. SGLT1 mediates the BBM Na^+/glucose cotransport [Vehyl et al., 1992; Vehyl et al., 1993; Weber et al., 1991], and GLUT2 mediates the facilitative Na^+-independent diffusion of glucose and fructose through the BLM [Kellet and Helliwell, 2000] as well as possibly through the BBM [Helliwell et al., 2000a, Helliwell et al., 2000b; Kellet, 2001]. Fructose transport is by facilitated diffusion mediated by GLUT5 in the BBM [Burant and Bell, 1992; Burant et al., 1992; Rand, 1993; Shu et al., 1997].

Systemically active glucocorticosteroids given by mouth enhance the intestinal absorption of sugars [Scott et al., 1980; Batt and Scott, 1982], and accelerate the development of the intestine in early life [Lebenthal et al., 1972]. The locally acting steroid budesonide is useful in the treatment of patients with Crohn's disease [Lofberg et al., 1993; Greenberg et al., 1994; Rutgeerts et al., 1994; Thiesen and Thomson,1996]. In young rats with an intact intestinal tract, budesonide enhances the intestinal uptake of fructose and some lipids [Thiesen et al., 1996]. While the injection of dexamethasone reduces the DNA content of the bowel following intestinal resection [Park et al., 1994], it is unknown what is its effect on nutrient absorption. Accordingly, this study was undertaken to test the hypothesis that glucocorticosteroids enhance the intestinal absorption of sugars following intestinal resection.

2) Methods and Materials

2.1) Western immunoblotting

BLM and BBM proteins from at least three animals in each group were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) using a modification of the method developed by Laemmli (1970). After their migration, proteins were immobilized on a solid support by electroblotting to a nitrocellulose membrane. Then, the membranes were blocked by incubation overnight in BLOTTO (Bovine Lacto Transfer Technique Optimizer) containing 5% w/v dry milk in Tween Tris Buffered Saline (TTBS: 0.5% Tween 20, 30mM Tris, 150 mM NaCl).

Membranes were washed three times with TTBS for at least 10 minutes. Then, membranes were probed with specific rabbit anti-rat antibodies that bound specifically to the antigens of interest. The incubation was carried out at room temperature for 2 hours for α1-Na^+/K^+ ATPase, β1-Na^+/K^+ ATPase, GLUT2, GLUT5, and was incubated overnight for SGLT1. The antibodies were diluted in 2% dry milk in TTBS at 1:500 for α1-Na^+/K^+ ATPase, GLUT2, GLUT5, SGLT1, and 1:2000 for β1-Na^+/K^+ ATPase.

The polyclonal antibodies against SGLT1 and GLUT2 were obtained from Biogenesis, Poole, England. The polyclonal antibody against GLUT5 was obtained from Chemicon International Inc., Temecula, California. The polyclonal antibodies anti-rat α1 and β1-Na^+/K^+ ATPase were obtained from Upstate Biotechnology Inc., Lake Placid, NY.

Following this primary incubation, the BBM and BLM were washed three times with TTBS to remove the residual unbound primary antibody. Membranes were then incubated for one hour with goat anti-rabbit antibody, conjugated with horseradish peroxidase (HRP) (Pierce, Rockfort, Illinois, USA) that bound the primary antibody. This second antibody was diluted at 1:20000 in 2% dry milk in TTBS.

After three washes in TTBS to remove residual secondary antibody, the membranes were incubated for 5 minutes with SuperSignal® Chemiluminescent-HRP

Substrate (Pierce, Rockfort, Illinois, USA) composed of 50% Stable Peroxide Solution and 50% of Luminol/Enhancer Solution. This reacted with the secondary antibody and made visible the antigens of interest. Then, membranes were exposed to X-OMAT AR films for various times, and were successively plunged into developer, water and fixer. The relative band densities were determined by transmittance densitometry, using a Bio-Rad imaging densitometer (Life science group, Cleveland, Ohio, USA).

2.2) Northern immunoblotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E. coli*) were transformed with plasmids containing the desired DNA sequences to be used to probe for the Northern blotting. SGLT1 cDNA probe was donated by Dr. Davidson, University of Chicago; cDNA probes encoding the α1 and β1 Na^+/K^+ ATPase subunit isoforms were obtained from Dr. Lingrel, University of Cincinnati; cDNA probes encoding GLUT5 and GLUT2 were obtained from Dr. Bell, University of Chicago; Early response genes (ERG) probes were obtained from Oncogene Research Products; the cDNA probe encoding proglucagon was obtained form Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; and the ODC was obtained from Dr. Blackshear, University of Chicago.

RNA was extracted from the jejunum and ileum of at least three animals in each of the four groups. These segments were homogenized in a denaturing solution containing guanidinium thiocyanate, using the biorad fast prep shaking centrifuge. Following acidification with 2 M sodium acetate, a phenol chloroform extraction was performed. The upper aqueous phase was transferred to a tube, and the RNA was precipitated with isopropanol and washed with 70% ethanol. RNA samples were stored at -70°C.

Equal amounts of total RNA were denatured in a sample loading buffer. Ethidium bromide (10 mg/ml) was added, so that the integrity of the RNA can be determined by visualizing the 28 S and 18 S ribosomal bands under UV light.

Total RNA was separated, based on molecular weight as it was electrophoresed through a denaturing agarose gel (1.16% agarose). RNA was transferred from the gel to a nylon membrane by capillary action overnight.

As a pre-hybridization, membranes were incubated for 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at the adequate temperature overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid) in order to reduce non-specific binding sites. They were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was done using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA), and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA). To determine the exact RNA quantity that had been loaded, the 28 S ribosomal band density on the membrane was evaluated after the transfer.

8) Results

3.1) Animal Characteristics

Body weight gain, food intake, and weight gain (g/day) per food intake (g/day) were similar in transected and in resected rats (Table 43). Dexamethasone reduced body weight gain in resected animals (from 4.8 g/day in controls to 2.0 g/day in those given dexamethasone), whereas budesonide and prednisone had no such effect (Table 44). None of the steroids influenced food intake.

The total weight of the intestine and the percentage of the intestinal wall comprised of scrapable mucosa were similar in the jejunum and ileum of resected and transected rats (Table 45). Similarly, in resected rats neither budesonide, prednisone nor dexamethasone had an effect on the weight of the intestine, or on the percentage of the intestinal wall comprised of mucosa. (Table 46). For this reason, the rates of sugar uptake were expressed on the basis of intestinal weight (nmol*100mg tissue $^{-1}$*min^{-1}).

3.2) Transporter Activities

There was no effect of the resection of the middle half of the intestine on the values of the maximal transport rate (Vmax) or the apparent Michaelis constant (Km) of the jejunal or ileal uptake of glucose (Table 47). However, the values of the Vmax and Km for jejunal uptake of glucose were both approximately 120% higher in rats given budesonide as compared with control vehicle (Table 48). The values of the Vmax and Km for jejunal uptake of glucose in resected rats were lower in those given prednisone or dexamethasone, as compared with budesonide. None of the steroids had an effect on the values of Vmax or Km for the ileal uptake of glucose. Resection had no effect on the rates of jejunal and ileal uptake of L-glucose (table 49). Similarly, steroids had no effect on L-glucose uptake into the jejunum (Table 50).

Resection had no effect on the uptake of fructose into the jejunum or ileum (Table 51). In the jejunum of resected rats, dexamethasone reduced the uptake of fructose by about 13% as compared with control vehicle, budesonide or prednisone (Table 52). In the ileum of resected rats, budesonide increased the uptake of fructose by 70%, as compared with control vehicle, prednisone or dexamethasone.

3.3) Transporter Protein Abundance and Expression of mRNA

The protein abundance SGLT1 and Na^+/K^+ ATPase alpha 1 and beta 1 in the jejunum was not altered by intestinal resection (Table 53). However, in the ileum the abundance of SGLT1 was increased by resection, whereas Na^+/K^+ ATPase alpha 1 was reduced and Na^+/K^+ ATPase beta 1 was unchanged. Resection did not change the jejunal or ileal mRNA expression of SGLT1 or Na^+/K^+ ATPase beta 1, but Na^+/K^+ ATPase alpha 1 mRNA expression in the jejunum was reduced (Table 54). In the ileum, budesonide and dexamethasone increased the abundance of Na^+/K^+ ATPase beta 1, as compared to the control vehicle and the prednisone group (Table 55). In animals with resection, steroids had no effect on the mRNA expression of SGLT1 or Na^+/K^+ ATPase in the jejunum and ileum (Table 56). Budesonide, prednisone or dexamethasone administrated to resected animals did not change the jejunal abundance of SGLT1, or either of the Na^+/K^+ ATPase subunits (Table 55).

Resection did not have any significant effect on the abundance of GLUT5 or GLUT2 (Table 57). The expression of GLUT5 mRNA was reduced in the jejunum of resected as compared to transected animals, and was unchanged in the ileum (Table 58).

GLUT2 mRNA was unchanged with resection. Steroids did not affect the protein abundance of either GLUT5 or GLUT2 (Table 59). Prednisone increased the mRNA expression of GLUT5 in the jejunum of resected animals, as compared to transected controls (Table 60). The expression of GLUT5 mRNA in the ileum, and GLUT2 mRNA in either jejunum and ileum, were unchanged by the administration of steroid.

3.4) Early Response Gene Expression

Animals undergoing intestinal resection showed no differences in the expression of the mRNAs for c-myc or c-jun, as compared to those undergone transection (Table 61). Budesonide, prednisone and dexamethasone reduced the jejunal expression of the mRNA of c-jun in resected animals (Table 62). No differences were observed in the jejunal expression of c-myc, or ileal expression of c-myc and c-jun.

3.5) Proglucagon and ODC Expression

The expression in the jejunum and ileum of the mRNA for proglucagon was not changed by resection. However, ODC expression in the jejunum of resected animals was reduced (Table 63). No changes in the ileal expression of the mRNA for ODC were observed. Steroids reduced the jejunal expression of the mRNA for proglucagon in resected animals, as compared with control vehicle but did not change the ileal expression (Table 64). Steroids had no effect on the jejunal or ileal expression of the mRNA for ODC.

9) Discussion

We chose a protocol of non-massive intestinal resection (50%) where the remaining proximal and distal intestinal remnants were adequate to assess the morphology and function at these sites [Keelan et al., 1996]. This 50% resection did not result in body weight loss, and is closer to the more common clinical situation seen, for example, in patients with Crohn's disease. In this study, the animals' food intake, body weight gain, intestinal weight, and the percentage of the intestinal weight comprised of mucosa were unaffected by intestinal resection. Thus, the alterations in the function of the non-resected intestine in animals given steroids were not due to changes in these end-points.

The intestinal absorption of nutrients is subject to the adaptation of mediated- and non-mediated processes, with alterations in transport influenced by the animal's age, diet, as well as by pathological processes such as diabetes mellitus, chronic ethanol intake, and abdominal irradiation [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, morphological and functional changes occur depending upon the extent of the intestine removed, the site studied, and the lipid content of the diet [Keelan et al., 1996]. The signals for this process are unknown, but may include proglucagon-derived peptides, early response genes (ERGs), and ornithine decarboxylase (ODC) [Bristol and Williamson, 1988; Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998; Reimer and McBurney, 1996; Jeppesen et al., 2001; Warner, 2001]. ERGs such as c-myc, c-jun and c-fos have been demonstrated to be involved in processes of intestinal proliferation and differentiation, as also is ODC, a key enzyme in the synthesis of polyamines which are a requirement in any proliferative event [Bristol and Williamson, 1988; Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998]. Proglucagon also contributes to the intestinal adaptive process [Mojsov et al., 1986; Orskov et al., 1987]. For example, the administration of short chain fatty acids increases proglucagon mRNA expression in rats undergoing intestinal resection [Tapenden and McBurney, 1998]. In this study, the adaptive response to intestinal resection did not change the expression of the mRNAs for ERGs or proglucagon, but ODC mRNA was reduced in the jejunum of resected rats. The adaptation in sugar absorption following intestinal resection must involve other signals. Epimorphin/syntaxin 2 mRNA that codes for a membrane-associated protein involved in morphogenesis of the lungs and skin, and PC4/TIS7, a gene involved in nerve growth factor-mediated cytodifferentiation, may be among other signals that might be involved in the adaptive response after intestinal resection [Goyal et al., 1998; Rubin et al., 1998]. New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001].

Glucocorticosteroids are used to treat patients with a variety of intestinal conditions, including Crohn's disease and ulcerative colitis. Clinical studies have focussed on the use of the potent locally acting steroid, budesonide [Lofberg et al., 1993; Greenberg et al., 1994; Rutgeerts et al., 1994; Thiesen and Thomson,1996]. Prednisone and budesonide modify the morphology and absorptive function of the intestine in young rats with an intact intestinal tract [Thiesen et al., 1996]. This study compares the influence of the locally active budesonide versus two systemically active steroids (prednisone given by mouth, and dexamethasone given by subcutaneous injection) in adult animals in which a portion of the jejunum and ileum remained after removal of the middle half of the small intestine.

Although we used the same dose of dexamethasone (128 µg/kg, subcutaneously) which has been reported previously to blunt the expected increase in the bowel content of DNA one week after an 80% enterectomy [Park et al., 1994], we were unable to demonstrate any adverse effect of dexamethasone on the weight of the jejunum or ileum of 50% resected animals. This lack of detrimental effect of dexamethasone may have been due to differences in animal strain, their age, the length of intestine resected (50% versus 80%), the time after resection when our studies were performed, or the total dose of dexamethasone used. In resected animals, neither budesonide nor prednisone altered the weight of the intestine, nor the percentage of the intestinal wall comprised of mucosa. It is possible that had we subjected the animals to a massive bowel resection (80% of small bowel removed), then the weight of the remaining intestine per unit length or surface area might have increased. Nonetheless, based on this data two weeks following a 50% enterectomy, it is clear that these steroids given in these doses and by these routes had no adverse effects on the weight of the intestine. Thus, the data suggest that the effects of steroids on the absorptive functions of the intestine, to be discussed, were not due to any steroid-induced change in the mass of the intestine.

While the absorption of some nutrients may be increased after intestinal resection, the magnitude of this effect depends on the extent of resection, the site and the time after surgery when uptake experiments are performed, as well as the manner of the expression of the data [Hanson et al., 1977; Williamson et al., 1978; Williamson, 1982a; Williamson, 1982b]. With the removal of the middle half of the small intestine in this study, there was no change in the uptake of D-glucose (Table 47), L-glucose or D-fructose (Tables 49 and 51), as compared with transected animals. We could have subjected the animals to a massive small bowel resection and potentially been able to show enhanced uptake of sugars. However, the interpretation of the results would then have been more difficult, because of the expected concomitant changes in food intake, body weight gain and intestinal weight. Furthermore, we wished these studies to have some potential clinical relevance. For example, in patients with Crohn's disease, prednisone or budesonide may be used therapeutically in doses similar to those used in this study, and while many of these individuals may have previously undergone an intestinal resection, massive resections would be unusual.

The doses of prednisone and budesonide used in this study were chosen on the basis of regimens which have been shown to be clinically useful in humans [Lofberg et al, 1993; Rutgeerts et al., 1994; Bratssand, 1990]. The dose used has been shown to modify intestinal absorption of fructose and some lipids in chow-fed rats with an intact intestine [Thiesen et al., 1996]. There is a difference in the potency of various steroids on the absorption of sugars after a 50% enterectomy: prednisone had no influence on the value of the Vmax of glucose uptake or on the uptake of fructose, whereas budesonide increased by over 120% the value of the Vmax for jejunal uptake of D-glucose (Table 48). As well, budesonide increased by 70% the ileal uptake of fructose (Table 52). This occurred without any change in the non-mediated passive component of sugar uptake, as measured with L-glucose (Table 50). Thus, the locally active steroid budesonide accelerates the intestinal absorption of these two sugars following intestinal resection.

The protein abundance and the mRNA expression of the transporters responsible for the brush border membrane uptake of glucose and fructose (SGLT1 and GLUT5 respectively) did not correlate with the changes in the activity of these transporters. For instance, the enhanced jejunal glucose uptake with budesonide (Table 48) was not accompanied by an increase in the abundance of SGLT1 or its mRNA (Tables 55 and 56). Also, the enhanced fructose uptake with budesonide in the ileum (Table 52) was not accompanied by changes in GLUT5 abundance or mRNA expression (Tables 57 and 58). We did not perform immunohistochemistry of SGLT1 or GLUT5, so it is possible that steroids may modify sugar uptake by changing the distribution of these transporters along the villus, thereby increasing uptake without any variation in the total abundance of transporters. Also, Kellett and colleagues have provided evidence to suggest that under some circumstances when the activity of SGLT1 is stimulated, the increased cytosolic levels of PKC βII facilitate the trafficking of GLUT2 to the BBM, thereby providing and additional transporter for glucose and fructose [Kellet and Helliwell, 2000; Helliwell et al., 2000a; Helliwell et al., 2000b; Kellet, 2001]. Thus, it is possible that a post-translational event is involved in the regulation of sugar uptake in response to the administration of steroids.

Although the systemic bioavailability of budesonide is approximately one order of magnitude lower than for prednisone, its potency is much higher [Lofberg et al., 1993; Rutgeerts et al., 1994; Thiesen et al., 1996; Brattsand, 1990]. We speculate that the stimulating effect of budesonide on the jejunal uptake of glucose by SGLT1 and the ileal uptake of fructose by GLUT5 is the result of its greater effect on the enterocyte receptors for glucocorticosteroids. This adaptive response with budesonide following intestinal resection may be important to maintain the well-being of the animal. The glucose and fructose absorption-promoting effect of budesonide following intestinal resection may prove to be a useful agent to enhance the intestinal adaptive response.

Table 43. Effect of Resection on Body Weight Gain and Food Consumption of Adult Rats Undergoing Intestinal Transection

Surgery	Weight Gain (g/day)	Food Intake (g/day)	Weight Gain per Food Intake(%)
Transected	6.3 ± 0.3	23.0 ± 1.2	27.8 ± 1.0
Resected	4.8 ± 0.9	20.7 ± 1.3	30.5 ± 4.1

Mean ± SEM

None of these differences was statistically significant.

Table 44. Effect of Steroids on Body Weight Gain and Food Consumption of Adult Rats Undergoing Intestinal Resection

Drug	Weight Gain (g/day)	Food Intake (g/day)	Weight Gain per Food Intake (%)
Control	4.8 ± 0.9	20.7 ± 1.3	30.5 ± 4.1
Budesonide	3.6 ± 0.5	20.0 ± 2.9	18.1 ± 1.3 *
Prednisone	4.4 ± 0.4	20.2 ± 0.4	24.5 ± 1.8 ~
Dexamethasone	2.0 ± 0.3 *	25.5 ± 2.7	6.9 ± 0.8 *~^

Mean ± SEM

*, $p<0.05$, budesonide (Bud), prednisone (Pred) or dexamethasone (Dex) vs control (Con)

~, $p<0.05$, prednisone (Pred) or dexamethasone (Dex) vs budesonide (Bud)

^, $p<0.05$, dexamethasone (Dex) vs prednisone (Pred)

Table 45. Effect of Resection on Characteristics of Intestine

Surgery		Intestinal Weight (mg/cm)	Intestinal Wall Comprised of Mucosa (%)
Transected	Jejunum	19.5 ± 3.0	51.6 ± 1.9
	Ileum	15.0 ± 2.5	52.7 ± 4.7
Resected	Jejunum	23.4 ± 3.7	47.5 ± 4.1
	Ileum	17.5 ± 4.8	46.4 ± 4.7

Mean ± SEM

None of these differences was statistically significant.

Table 46. Effect of Steroids on Characteristics of Intestine of Rats Undergoing Intestinal Resection

Site	Drug	Intestinal Weight (mg/cm)	Intestinal Wall Comprised of Mucosa (%)
Jejunum	Control	23.4 ± 3.7	47.5 ± 4.1
	Budesonide	23.1 ± 2.3	48.7 ± 4.1
	Prednisone	24.2 ± 1.7	48.5 ± 7.2
	Dexamethasone	21.1 ± 2.5	64.2 ± 2.4
Ileum	Control	17.5 ± 4.8	46.4 ± 4.7
	Budesonide	14.5 ± 0.7	40.4 ± 6.0
	Prednisone	13.5 ± 1.7	52.2 ± 2.0
	Dexamethasone	9.4 ± 1.8	52.2 ± 2.1

Mean ± SEM

None of these differences was statistically significant.

Table 47. Effect of Resection on the Value of the Maximal Transport Rate (Vmax) and the Michaelis Affinity Constant (Km) of D-Glucose Uptake

	Jejunum		Ileum	
	Vmax	Km	Vmax	Km
Transected	2986 ± 392	40.8 ± 10.3	5083 ± 1043	134 ± 37.4
Resected	3066 ± 217	39.7 ± 5.5	2726 ± 320	59.7 ± 11.9

Mean ± SEM

None of these differences was statistically significant

Vmax (Maximal Transport Rate), nmol*100 mg $tissue^{-1}$*min^{-1}

Km (Michaelis Affinity Constant), mM

Table 48. Effect of Steroids on the Value of the Maximal Transport Rate (Vmax) and the Michaelis Affinity Constant (Km) of D-glucose in the Jejunum and Ileum of Rats Undergoing Intestinal Resection

	Jejunum		Ileum	
Drug	Vmax	Km	Vmax	Km
Control	3066 ± 217	39.7 ± 5.5	2726 ± 320	59.7 ± 11.9
Budesonide	6790 ± 803 *	93.7 ± 16.5 *	5091 ± 964	131.1 ± 34.0
Prednisone	2531 ± 535 ~	35.7 ± 15.2 ~	9354 ± 3313	239.3 ± 102.8
Dexamethasone	2991 ± 283 ~	39.3 ± 7.3 ~	5062 ± 2464	118.3 ± 80.7

Mean ± SEM

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

Vmax (Maximal Transport Rate), nmol*100 mg tissue^{-1}*min^{-1}

Km (Michaelis Affinity Constant), mM

Table 49. Effect of Resection on the Rate of Uptake of L-Glucose

Surgery	Jejunum	Ileum
Transected	25.9 ± 2.2	26.4 ± 2.2
Resected	24.1 ± 1.6	23.6 ± 2.9

Mean ± SEM

The rates of uptake of L-glucose are expressed as nmol*100 mg $tissue^{-1}$*min^{-1}*mM^{-1}.

None of these differences was statistically significant.

Table 50. Effects of Steroids on the Rate of Uptake of L-Glucose

Drug	Jejunum	Ileum
Control	24.1 ± 1.6	23.6 ± 2.9
Budesonide	27.7 ± 2.4	24.2 ± 2.9
Prednisone	25.8 ± 2.5	27.0 ± 2.7
Dexamethasone	20.0 ± 1.4	25.0 ± 2.4

Mean ± SEM

The rates of uptake of L-glucose are expressed as nmol*100 mg tissue^{-1}*min^{-1}*mM^{-1}.

None of these differences was statistically significant.

Table 51. Effect of Resection on the Rate of Uptake of D-Fructose

Surgery	Jejunum	Ileum
Transection	34.4 ± 1.9	26.6 ± 2.0
Resection	31.9 ± 1.5	26.5 ± 2.8

Mean ± SEM

The rates of uptake of D-fructose are expressed as nmol*100 mg tissue^{-1}*min^{-1}. This represents the slope of the linear relationship between fructose concentrations and uptake.

None of these differences was statistically significant.

Table 52. Effect of Steroids on the Rate of Uptake of D-Fructose in Rats Undergoing Intestinal Resection

Drug	Jejunum	Ileum
Control	31.9 ± 1.5	26.5 ± 2.8
Budesonide	30.4 ± 2.3	45.0 ± 3.8 *
Prednisone	33.5 ± 2.3	27.3 ± 1.9 ~
Dexamethasone	23.3 ± 1.4 ~^*	23.2 ± 1.5 ~

Mean ± SEM

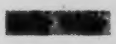
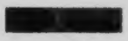
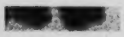
The rates of uptake of D-fructose are expressed as nmol*100 mg $tissue^{-1}$*min^{-1}*. This represents the slope of the linear relationship between fructose concentration and uptake.

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

Table 53. Effect of Resection on the Expression of SGLT1 and Na$^+$/K$^+$-ATPase Alpha 1 and Beta1 Subunits

	Jejunum			Ileum		
	SGLT1	Alpha 1	Beta 1	SGLT1	Alpha 1	Beta 1
						
	R T	R T	R T	R T	R T	R T
	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	1.08 ± 0.06	1.19 ± 0.35	1.72 ± 0.58	1.24 ± 0.07#	0.64 ± 0.18#	0.63 ± 0.48

Mean ± SD

SGLT1, the sodium-dependent transporter in the brush border membrane

Alpha 1, the Na$^+$/K$^+$ ATPase α subunit in the basolateral membrane

Beta 1, the Na$^+$/K$^+$ ATPase β subunit in the basolateral membrane

#, $p<0.05$, resected vs transected

Table 54. Effect of Resection on the mRNA Expression of SGLT1 and Na^+/K^+-ATPase Alpha 1 and Beta1 Subunits

	Jejunum			Ileum		
	SGLT1	Alpha 1	Beta 1	SGLT1	Alpha 1	Beta 1
	▬	▬	▬	▬	▬	▬
	R T	R T	R T	R T	R T	R T
	n = 5	n = 4	n = 4	n = 3	n = 4	n = 5
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	0.85 ± 0.38	0.59 ± 0.28#	0.63 ± 0.29	1.05 ± 0.93	0.96 ± 0.24	0.91 ± 0.24

Mean ± SD

SGLT1, the sodium-dependent transporter in the brush border membrane

Alpha 1, the Na^+/K^+ ATPase α subunit in the basolateral membrane

Beta 1, the Na^+/K^+ ATPase β subunit in the basolateral membrane

#, $p<0.05$, resected vs transected

Table 55. Effect of Steroids on the Expression of SGLT1 and Na$^+$/K$^+$-ATPase Alpha 1 and Beta1 Subunits

	Jejunum			Ileum		
Drug	SGLT1	Alpha 1	Beta 1	SGLT1	Alpha 1	Beta 1
	D P B C	D P B C	D P B C	D P B C	D P B C	D P B C
	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Budesonide	0.66 ± 0.65	0.82 ± 0.15	1.02 ± 0.07	0.91 ± 0.37	4.22 ± 2.55	1.39 ± 0.02*
Prednisone	0.73 ± 0.51	0.40 ± 0.51	1.09 ± 1.28	1.18 ± 0.19	2.00 ± 2.25	0.93 ± 0.19~
Dexamethasone	1.08 ± 0.15	0.45 ± 0.62	0.89 ± 0.98	0.79 ± 0.20	3.44 ± 1.15	1.29 ± 0.03*^

Mean ± SD

SGLT1, the sodium-dependent transporter in the brush border membrane

Alpha 1, the Na$^+$/K$^+$ ATPase α subunit in the basolateral membrane

Beta 1, the Na$^+$/K$^+$ ATPase β subunit in the basolateral membrane

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

Table 56. Effect of Steroids on the mRNA Expression of SGLT1 and Na$^+$/K$^+$-ATPase Alpha 1 and Beta1 Subunits

	Jejunum			Ileum		
Drug	**SGLT1**	**Alpha 1**	**Beta 1**	**SGLT1**	**Alpha 1**	**Beta 1**
	D P B C	D P B C	D P B C	D P B C	D P B C	D P B
	n = 5	n = 4	n = 4		n = 4	C n = 5
				n = 3		
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Budesonide	0.80 ± 0.53	1.34 ± 0.72	1.24 ± 0.72	0.87 ± 0.37	0.83 ± 0.17	0.86 ± 0.12
Prednisone	1.31 ± 1.24	2.20 ± 2.06	2.33 ± 2.53	1.12 ± 0.50	0.96 ± 0.12	0.97 ± 0.11
Dexamethasone	0.83 ± 0.63	1.08 ± 0.48	1.18 ± 0.59	0.79 ± 0.03	0.88 ± 0.11	0.85 ± 0.17

Mean ± SD

SGLT1, the sodium-dependent transporter in the brush border membrane

Alpha 1, the Na$^+$/K$^+$ ATPase α subunit in the basolateral membrane

Beta 1, the Na$^+$/K$^+$ ATPase β subunit in the basolateral membrane

None of these differences was statistically significant.

Table 57. Effect of Resection on the Expression of GLUT5 and GLUT2

	Jejunum		Ileum	
	GLUT5	GLUT2	GLUT5	GLUT2
	T R	T R	T R	T R
	n = 3	n = 3	n = 3	n = 3
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	1.05 ± 0.26	1.77 ± 0.95	0.94 ± 0.08	1.40 ± 0.56

Mean ± SD

GLUT5, the sodium-independent fructose transporter in the brush border membrane

GLUT2, the sodium-independent glucose and fructose transporter in the basolateral membrane and in the brush border membrane

None of these differences was statistically significant.

Table 58. Effect of Resection on the mRNA Expression of GLUT5 and GLUT2

	Jejunum		Ileum	
	GLUT5	GLUT2	GLUT5	GLUT2
	R T	R T	R T	R T
	n = 3	n = 4	n = 3	n = 4
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	0.77 ± 0.05+	0.71 ± 0.30	0.81 ± 0.23	0.93 ± 0.14

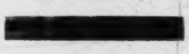
Mean ± SD

GLUT5, the sodium-independent fructose transporter in the brush border membrane

GLUT2, the sodium-independent glucose and fructose transporter in the basolateral membrane and in the brush border membrane

+, p<0.05, resected vs transected

Table 59. Effect of Steroids on the Expression of GLUT5 and GLUT2

	Jejunum		Ileum	
Drug	GLUT5	GLUT2	GLUT5	GLUT2
				
	D P B C	D P B C	D P B C	D P B
	n = 3	n = 3	n = 3	C
				n = 3
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Budesonide	1.69 ± 1.21	0.92 ± 0.30	0.74 ± 0.65	0.96 ± 0.33
Prednisone	1.50 ± 1.22	0.91 ± 0.52	0.85 ± 0.16	1.72 ± 0.74
Dexamethasone	1.43 ± 1.03	0.91 ± 0.24	0.74 ± 0.18	2.02 ± 0.94

Mean ± SD

GLUT5, the sodium-independent fructose transporter in the brush border membrane

GLUT2, the sodium-independent glucose and fructose transporter in the basolateral membrane and in the brush border membrane

None of these differences was statistically significant.

Table 60. Effect of Steroids on the mRNA Expression of GLUT5 and GLUT2

	Jejunum		Ileum	
Drug	GLUT5	GLUT2	GLUT5	GLUT2
	D P B C	D P B C	D P B	D P B C
	n = 3	n = 4	C	n = 4
			n = 3	
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Budesonide	1.18 ± 0.05	1.03 ± 0.60	0.89 ± 0.01	0.83 ± 0.17
Prednisone	2.21 ± 0.27*~	1.96 ± 2.15	0.87 ± 0.01	0.96 ± 0.12
Dexamethasone	1.09 ± 0.01^	0.94 ± 0.46	0.98 ± 0.25	0.88 ± 0.11

Mean ± SD

GLUT5, the sodium-independent fructose transporter in the brush border membrane

GLUT2, the sodium-independent glucose and fructose transporter in the basolateral membrane and in the brush border membrane

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

Table 61. Effect of Resection on the ERG Expression

	Jejunum			Ileum		
	c-myc n = 3	c-jun n = 3	c-fos	c-myc n = 3	c-jun n = 3	c-fos
Transected	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0	N.S.
Resected	0.77 ± 0.35	0.63 ± 0.45	N.S.	0.94 ± 0.19	0.83 ± 1.09	N.S.

Mean ± SD

None of these differences was statistically significant.

N. S. = no signal

Table 62. Effect of Steroids on the ERG Expression

	Jejunum			Ileum		
Drug	c-myc	c-jun	c-fos	c-myc	c-jun	c-fos
	n = 3	n = 3		n = 3	n = 3	
Control	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0	N.S.
Budesonide	1.08 ± 0.70	0.43 ± 0.02*	N.S.	0.83 ± 0.23	0.99 ± 0.54	N.S.
Prednisone	1.84 ± 1.83	0.46 ± 0.02*	N.S.	1.22 ± 0.48	1.42 ± 1.00	N.S.
Dexamethasone	1.03 ± 0.79	0.54 ± 0.11*	N.S.	1.06 ± 0.14	1.02 ± 0.18	N.S.

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

N.S.= no signal

Table 63. Effect of Resection on the Expression of Proglucagon and ODC

	Jejunum		Ileum	
	Proglucagon	ODC	Proglucagon	ODC
	n = 3	n = 3	n = 3	n = 4
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	1.10 ± 0.09	0.52 ± 0.12+	0.98 ± 0.11	0.85 ± 0.16

Mean ± SD

+, $p<0.05$, resected vs transected

Table 64. Effect of Steroids on the Expression of Proglucagon and ODC

	Jejunum		Ileum	
Drug	Proglucagon	ODC	Proglucagon	ODC
	n = 3	n = 3	n = 3	n = 3
Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Budesonide	0.49 ± 0.08*	1.02 ± 0.49	1.01 ± 0.10	0.81 ± 0.20
Prednisone	0.46 ± 0.08*	1.12 ± 0.72	1.01 ± 0.18	0.84 ± 0.11
Dexamethasone	0.44 ± 0.01*	0.78 ± 0.24	0.88 ± 0.22	0.82 ± 0.05

Mean ± SD

*, p<0.05, budesonide, prednisone or dexamethasone vs control

~, p<0.05, prednisone or dexamethasone vs budesonide

^, p<0.05, dexamethasone vs prednisone

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L) INTESTINAL RESECTION- AND STEROID-ASSOCIATED ALTERATIONS IN GENE EXPRESSION WERE NOT ACCOMPANIED BY CHANGES IN LIPID UPTAKE

1) Introduction

The topic of intestinal adaptation has been reviewed [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, there is hyperplasia of the remaining intestine, which may be accompanied by enhanced uptake of nutrients [Dowling and Booth, 1967; Weiser, 1973; Kinter and Wilson, 1975; Garvey et al., 1976; Hanson et al., 1977; King et al., 1981]. The signals which mediate this adaptive process are incompletely understood, but may include proglucagon-derived peptides, ornithine decarboxylase (ODC), and early response genes (ERG) [Bristol and Willianson, 1988; Bloom and Polak, 1982; Rountree et al., 1992; Tappenden and McBurney, 1998; Niot et al., 1997]. Epimorphin/syntaxin 2 mRNA, that codes for a membrane-associated protein involved in morphogenesis of the lungs and skin, as well as PC4/TIS7, a gene involved in nerve growth factor-mediated cytodifferentiation, may be among other signals that might also be involved in the adaptive response after intestinal resection [Rubin et al., 1998; Goyal et al., 1998]. New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001].

Systemically active glucocorticosteroids given by mouth enhance the intestinal absorption of sugars [Scott et al., 1980; Batt and Scott, 1982], and accelerate the development of the intestine in early life [Lebenthal et al., 1972]. The locally acting steroid budesonide has been shown to be useful in the treatment of patients with Crohn's disease [Lofberg et al., 1993; Greenberg et al., 1994; Rutgeerts et al., 1994; Campieri et al., 1995; Thiesen and Thomson, 1996; Greenberg, 1994]. In young rats with an intact intestinal tract, budesonide enhances the intestinal uptake of fructose and some lipids [Thiesen et al., 1996]. While the injection of dexamethasone reduces the DNA content of the bowel following intestinal resection [Park et al., 1994], it is unknown what is its effect on nutrient absorption.

Intestinal lipid uptake occurs by a process of passive permeation [Poirier et al., 1996], but a component of the uptake of long-chain fatty acids is also mediated by the sodium/hydrogen exchanger and/or by fatty acid binding proteins in the brush border membrane or in the cytosol of the enterocyte, such as the liver fatty acid binding protein (L-FABP) and the ileal lipid binding protein (ILBP) [Schoeller et al., 1995a; Schoeller et al., 1995b; Poirier et al., 1996; Niot et al., 1997; Storch, 2001]. It is unknown if the fatty acid binding proteins in the intestine are influenced by steroids or by intestinal resection, and whether these are associated with alterations in lipid uptake.

Accordingly, these studies were undertaken to test the hypotheses that 1) the desired intestinal adaptive response following intestinal resection may be enhanced further by the administration of the locally active steroid budesonide, or the systemically active steroids prednisone or dexamethasone; and 2) any alterations in lipid uptake is associated with changes in the expression of selected signals as well as the expression of the mRNAs for L-FABP and ILBP.

2) Methods and Materials

2.1) Northern Immunoblotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E.coli*) were transformed with plasmids containing the desired DNA sequences to be used as a probe for the Northern blotting. Early Response Gene (ERG) probes were obtained from Oncogene Research Products; the cDNA probe encoding proglucagon was obtained from Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; the ODC probe was obtained from Dr. Blackshear, University of Chicago; L-FABP and ILBP probes were provided by Dr. Agellon, University of Alberta. A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labelled DNA (Roche Diagnostics, Quebec, CA) that was dotted on the same membrane.

RNA was extracted from the jejunum and ileum of at least three animals in each of the four groups. These segments were homogenized in a denaturing solution containing guanidinium thiocyanate, using the biorad fast prep shaking centrifuge. Following acidification with 2 M sodium acetate, a phenol chloroform extraction was performed. The upper aqueous phase was transferred to a tube, and the RNA was precipitated with isopropanol and washed with 70% ethanol. RNA samples were stored at -70°C.

Total RNA was separated, based on molecular weight as it was electrophoresed through a denaturing agarose gel (1.16% agarose). RNA was then transferred from the gel to a nylon membrane by capillary action overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA onto the membranes.

As a pre-hybridization, membranes were incubated for 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at 60 ^{0}C overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid). They were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was done using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA), and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA). To determine the exact RNA quantity that had been loaded, the 28 S ribosomal band density on the membrane was evaluated after the transfer.

3) Results

3.1) Animal Characteristics

Body weight gain, food intake and weight gain per food intake were similar in transected and in resected rats (Table 43). Dexamethasone reduced body weight gain in resected animals, whereas budesonide and prednisone had no effect (Table 44). None of the steroids influenced the animals‘ food intake. However, the weight gain (g/day) per food intake (g/day) was approximately 30% lower in animals given budesonide as compared with control, and was about 77% lower in animals given dexamethasone (Table 2).

The total weight of the intestine and the percentage of the intestinal wall comprised of scrapable mucosa were similar in the jejunum and ileum of resected and transected rats (Table 45). Similarly, in resected rats neither budesonide, prednisone nor dexamethasone had an effect on the weight of the intestine, or on the percentage of the intestinal wall comprised of mucosa (Table 46). For this reason the rates of sugar and lipid uptake were expressed on the basis of 100 mg of intestinal tissue.

3.2) Lipids

Neither intestinal resection (Table 65) nor steroids (Table 66) influenced the rate of uptake of 16:0, 18:2 or cholesterol into the jejunum or ileum.

3.3) Early Response Gene Expression (ERG)

Animals undergoing intestinal resection had no differences in the expression of the mRNAs for the ERGs (c-myc and c-jun) as compared to those undergoing intestinal transection (Table 61). Budesonide, prednisone and dexamethasone reduced the jejunal expression of c-jun in resected animals (Table 62). No differences were observed in the expression of jejunal c-myc or ileal c-myc or c-jun.

3.4) Proglucagon and ODC Expression

Proglucagon mRNA expression in the jejunum and ileum was not changed by intestinal resection (Table 63). Expression of the mRNA for ODC in the jejunum of resected animals was reduced ($p<0.05$). No changes were observed in the ileal expression of the mRNA for ODC.

All three steroids reduced the jejunal expression of proglucagon in resected animals, but did not change the ileal expression (Table 64). ODC mRNA in either jejunum and ileum was also not changed by resection (Table 63) or by steroids (Table 64).

3.5) mRNA Expression of L-FABP and ILBP

As expected, the ileal lipid binding protein (ILBP) mRNA was present only in the ileum. Its abundance was unaffected by steroids or by resection (Table 67 and 68). The liver fatty acid binding protein (L-FABP) mRNA was also unaffected by steroids, but resection reduced the jejunal expression of L-FABP mRNA (Table 67 and 68).

5) Discussion

The intestinal absorption of nutrients is subject to adaptation of mediated- and non-mediated processes, with changes in transport influenced by the animal's age, diet, as well as by pathological processes such as diabetes mellitus, chronic ethanol intake, and abdominal irradiation [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, morphological and functional changes occur depending upon the extent of the intestine removed, the site studied, and the lipid content of the diet [Keelan et al., 1996]. The signal for this process is unknown, but may include proglucagon-derived peptides, early response genes (ERG) and ornithine decarboxylase (ODC) [Bristol and Williamson, 1988; Bloom and Polak, 1982; Reimer and McBurney, 1996; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998]. In thi study, the intestinal adaptive response to intestinal resection did not change the expression of ERG or proglucagon (Table 7 and 9), but ODC was reduced in the jejunum of resected rats. It is unlikely that this is an important signal for the adaptation of lipid uptake, since the uptake of lipids was unchanged (Table 65).

While the absorption of some nutrients may be increased after intestinal resection, the magnitude of this effect depends on the extent of resection, the site and the time after surgery when uptake experiments are performed, as well as on the manner of expression of the data [Hanson et al., 1977; Williamson et al., 1978; Williamson, 1982a; Williamson, 1982b]. With the removal of the middle half of the small intestine in this study, there was no change in the rate of uptake of lipids (Table 65). We could have subjected the animals to a massive small bowel resection and potentially been able to show enhanced uptake of lipids. However, the interpretation of the results would then have been made difficult because of the expected concomitant changes in food intake, body weight gain and intestinal weight. Furthermore, we wished these studies to have some potential clinical relevance. For example, in patients with Crohn's disease, prednisone or budesonide may be used therapeutically in the doses used in this study, and while these individuals may have previously undergone on intestinal resection, massive resections would be unusual.

Glucocorticosteroids are used to treat patients with a variety of intestinal conditions, including Crohn's disease and ulcerative colitis. Recent clinical attention has focused on the use of the potent locally acting steroid, budesonide [Lofberg et al., 1993; Greenberg et al., 1994; Rutgeerts et al., 1994; Campieri et al., 1995; Thiesen and Thomson, 1996]. Prednisone and budesonide modify the morphology and absorptive function of the intestine in young rats with an intact intestinal tract [Thiesen et al., 1996]. This study compares the influence of the locally active budesonide versus two systemically active steroids (prednisone given by mouth, and dexamethasone given by subcutaneous injection) in adult animals in which a portion of the jejunum and ileum remained after removal of the middle half of the small intestine.

The doses of prednisone and budesonide used in this study were chosen on the basis of regimens which have been shown to be useful clinically [Greenberg et al., 1994; Rutgeerts et al., 1994; Bratssand, 1990]. The dose used in this study has been shown to modify intestinal absorption of some lipids in chow-fed rats with an intact intestine [Thiesen and Thomson, 1996]. None of the three steroids affected the jejunal or ileal uptake of palmitic or linoleic acids or cholesterol (Table 66). Adaptation of intestinal uptake of lipids is associated with changes in the intestinal brush border membrane (BBM) and enterocyte microsomal membrane phospholipids or phospholipid fatty acids [Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1986; Keelan et al., 1987; Keelan et al., 1989; Keelan et al., 1993], as well as phospholipid and fatty acid metabolizing enzymes [Garg et al., 1988; Garg et al., 1990]. The only exception to this general rule has been the lack of effect of variations in dietary lipids on the phospholipids in BBM, which under the same experimental conditions do demonstrate changes in lipid uptake [Keelan et al., 1987].

In this study we did not measure BBM lipids, so we are not able to comment upon whether the absence of an effect of steroids on fatty acid uptake was due to their lack of effect on BBM lipids or on the protein-mediated components of uptake [Schoeller et al., 1995a; Schoeller et al., 1995b; Poirier et al., 1996; Ibrahimi et al., 1996; Keelan et al., 1996]. There are lipid binding proteins in the intestine [Schoeller et al., 1995a; Schoeller et al., 1995b; Poirier et al., 1996; Niot et al., 1997; Storch, 2001], but L-FABP is unlikely to play a major rule, as suggested by the marked decline in jejunal L-FABP mRNA (Table 67), but no alteration in lipid uptake (Table 65). Recent support for the uncoupling between intestinal lipid uptake and lipid binding proteins comes from the findings that 1) clones of mouse with disrupted genes for the intestinal fatty acid binding protein (I-FABP) are viable, have elevated plasma triacylglycerol and weigh more than the wild type [Vassielva et al., 2000]; and 2) dietary and diabetic alterations in lipid uptake are not paralleled by similar alterations in lipid binding protein expression [Drozodwski et al., 2001]. Our findings support this general concept. We add the cautiounary comment, however, that we did not measure the BBM protein content of L-FABP and ILBP, and there may as well be other lipid binding proteins which we did not measure.

The systemic administration of dexamethasone blunts the morphological adaptation of the intestine of rats when they are studied one week after an 80% enterectomy [Park et al., 1994]. We used adult rats studied two weeks after a 50% enterectomy. We chose a protocol of non-massive resection which results in morphological and functional adaptation [Keelan et al., 1996], and in which the remaining proximal and distal intestinal remnants are adequate to assess the morphology and function at these sites. Furthermore, 50% resection does not result in body weight loss (Table 43), and is closer to the more common clinical situation seen, for example, in patients with Crohn's disease. In this study, the animals' food intake, body weight gain, intestinal weight, and the percentage of the intestinal weight comprised of mucosa were unaffected by intestinal resection (Table 43 and 45). Thus, the alterations in the function of the resected intestine in animals given steroids were not due to changes in these parameters.

Although we used the same dose of dexamethasone (128 μg/kg, subcutaneously) which has been reported previously to blunt the expected morphological response to intestinal resection [Park et al., 1994], we did not demonstrate any adverse effect of dexamethasone on the weight of the jejunum or ileum of resected animals (Table 46). This lack of detrimental effect of dexamethasone may have been due to differences in animal strain, their age, the length of intestine resected, the time after resection when our studies were performed, or the total dose of dexamethasone that was used. Neither budesonide nor prednisone altered the weight of the intestine, or the percentage of the intestinal wall comprised of mucosa in resected animals (Table 46). It is possible that had we subjected the animals to a massive bowel resection (80% of small bowel removed), then the weight of the remaining intestine per unit length or surface area might have changed. Nonetheless, based on this data two weeks following a 50% enterectomy, it is clear that these steroids given in these doses and by these routes have no adverse effects on the weight of the intestine.

The resection-associated alterations in the mRNA expression of ODC and I-FABP, and the steroid-associated changes in the mRNA expression of c-jun and proglucagon (Tables 63,67 and 64) were not accompanied by variations in the uptake of lipids (Tales 65 and 66). We suggest that these alterations in gene expression were insignificant to lead to an adaptation of lipid uptake. It is unclear what is the role of the altered expression of these genes.

Table 65. Effect of Resection on Uptake of Fatty Acids and Cholesterol

Surgery		Jejunum	Ileum
Transected			
	16:0	29.0 ± 3.2	20.0 ± 2.2
	18:2	28.6 ± 3.0	18.4 ± 2.1
	Chol	14.4 ± 1.8	12.6 ± 1.4
Resected			
	16:0	32.6 ± 4.9	24.8 ± 4.1
	18:2	28.4 ± 3.1	13.3 ± 1.4
	Chol	10.2 ± 2.0	9.2 ± 2.8

Mean ± SEM

The rates of uptake of lipids are expressed as nmol*100 mg tissue^{-1}*min^{-1}*mM^{-1}.

None of these differences was statistically significant.

16:0, palmitic acid; 18:2, linoleic acid; chol, cholesterol

Table 66. Effect of Steroids on Uptake of Fatty Acids and Cholesterol In Resected Rats

Site and Lipid	Control	Budesonide	Prednisone	Dexamethasone
Jejunum				
16:0	32.6 ± 4.9	21.8 ± 2.8	30.3 ± 3.4	19.3 ± 1.9
18:2	28.4 ± 3.1	27.3 ± 2.6	17.1 ± 2.8	29.2 ± 3.2
Chol	10.2 ± 2.0	8.0 ± 1.4	9.2 ± 2.6	10.6 ± 1.4
Ileum				
16:0	24.8 ± 4.1	17.4 ± 2.1	20.8 ± 3.7	21.9 ± 2.4
18:2	13.3 ± 1.4	20.2 ± 3.6	16.4 ± 3.1	17.5 ± 2.9
Chol	9.2 ± 2.8	12.4 ± 2.0	11.4 ± 2.0	10.8 ± 1.2

Mean ± SEM

The rates of uptake of lipids are expressed as nmol*100 mg $tissue^{-1}$*min^{-1}*mM^{-1}.

None of these differences was statistically significant.

16:0, palmitic acid; 18:2, linoleic acid; chol, cholesterol

Table 67. Effect of Resection on the the L-FABP and ILBP mRNA Expression

	Jejunum		Ileum	
Diet	L-FABP	ILBP	L-FABP	ILBP
Transected				
	1 ± 0	N.S.	1 ± 0	1 ± 0
Resected				
	0.60 ± 0.14+	N.S.	0.74 ± 0.09	0.86 ± 0.15

Mean ± SD

+, p<0.05, transection vs resection

N.S. no signal

Table 68. Effect of Steroids on the L-FABP and ILBP mRNA Expression

Diet	Drug	Jejunum		Ileum	
		L-FABP	ILBP	L-FABP	ILBP
Chow	Control	1 ± 0	N.S.	1 ± 0	1 ± 0
	Budesonide	1.06 ± 0.37	N.S.	0.91 ± 0.17	0.88 ± 0.21
	Prednisone	1.39 ± 0.90	N.S.	1.20 ± 0.29	1.05 ± 0.16
	Dexamethasone	1.06 ± 0.19	N.S.	0.98 ± 0.22	1.08 ± 0.24
Saturated	Control	1 ± 0	N.S.	1 ± 0	1 ± 0
	Budesonide	3.82 ± 5.00	N.S.	0.74 ± 0.30	1.01 ± 0.12
	Prednisone	4.68 ± 5.95	N.S.	0.65 ± 0.22	0.96 ± 0.17
	Dexamethasone	3.84 ± 4.88	N.S.	0.79 ± 0.12	0.91 ± 0.15
Polyunsaturated	Control	1 ± 0	N.S.	1 ± 0	1 ± 0
	Budesonide	0.98 ± 0.68	N.S.	0.96 ± 0.21	1.32 ± 0.67
	Prednisone	0.84 ± 0.56	N.S.	2.05 ± 1.49	2.37 ± 1.73
	Dexamethasone	0.91 ± 0.58	N.S.	1.03 ± 0.32	1.13 ± 0.31

Mean ± SD

None of these differences was statistically significant

N.S. no signal

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M) DIETARY LIPIDS ALTER THE EFFECT OF STEROIDS ON THE TRANSPORT OF SUGARS FOLLOWING INTESTINAL RESECTION IN RATS

1) Introduction

The topic of intestinal adaptation has been reviewed [Thomson et al., 1996; Thomson and Wild 1997]. Following intestinal resection, there is hyperplasia of the remaining intestine which may be accompanied by enhanced uptake of nutrients [Dowling and Booth, 1967; Garvey et al., 1976; Hanson et al., 1977; King et al., 1981; Kinter and Wilson, 1975; Weiser, 1973]. The signals which mediate this adaptive process are incompletely understood, but may include proglucagon-derived peptides, ornithine decarboxylase (ODC) and early response genes (ERG) [Bloom and Polak, 1982; Bristol and Williamson, 1988; Rountree et al., 1992; Sagor et al., 1983; Tappenden et al., 1998], and may be influenced by dietary lipids [Keelan et al, 1996; Tappenden et al., 1996]. Epimorphin/syntaxin 2 mRNA that codes for a membrane-associated protein involved in morphogenesis of the lungs and skin, and PC4/TIS7, a gene involved in nerve growth factor-mediated cytodifferentiation, may be among other signals that might be involved in the adaptive response after intestinal resection [Goyal et al., 1998; Rubin et al., 1998]. New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001]. In fact, isocaloric modification of the type of lipids in the diet is associated with changes in nutrient uptake in other models of intestinal adaptation such as diabetes, abdominal irradiation, chronic intake of ethanol, or aging [Thomson et al., 1996; Garg et al., 1988; Garg et al., 1990; Keelan et al., 1986; Keelan et al., 1989; Keelan et al., 1987;

Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1993; Suckling and Stange, 1985]. For example, sugar and lipid uptake is increased in rats fed an isocaloric saturated as compared with a polyunsaturated diet [Thomson et al., 1986].

Systemically active glucocorticosteroids given by mouth enhance the intestinal absorption of sugars [Batt and Scott; 1982; Scott et al., 1980], and accelerate the early development of the intestine [Lebenthal et al., 1972]. The locally acting steroid budesonide is useful to treat patients with Crohn's disease [Brattsand, 1990; Campieri et al., 1995; Greenberg et al., 1994; Lofberg et al., 1993; Rutgeerts et al., 1994; Thiesen and Thomson, 1996]. In young rats with an intact intestinal tract, budesonide enhances the intestinal uptake of fructose and some lipids [Thiesen and Thomson, 1996b]. While the injection of dexamethasone alters the DNA content of the bowel following intestinal resection [Park et la., 1994], it is unknown what is its effect on nutrient absorption.

The Na^+ -gradient across the intestinal brush border membrane (BBM) provides the driving force for glucose transport [Ricklis and Quastel, 1958]. This Na^+ -gradient is maintained by the action of the Na^+/K^+-ATPase in the basolateral membrane of the enterocyte [Hirayama et al., 1992]. SGLT1 mediates the BBM Na^+/glucose cotransport [Diamond and Karasov, 1984; Vehyl et al., 1992; Vehyl et al., 1993; Weber et al., 1991], and GLUT2 mediates the facilitative Na^+-independent diffusion of glucose, and fructose through the BLM [Caspary et al., 1968]. Recent evidence suggests that GLUT2 may also be in the BBM [Helliwell et al., 2000; Helliwell et al., 2000; Kellet et al., 2001]. Fructose transport is by sodium-independent facilitated diffusion mediated by GLUT5 in the BBM [Burant and Bell, 1992; Burant et al., 1992a; Burant et al., 1992b; Rand et al., 1993; Shu et al., 1997].

These studies were undertaken to test the hypothesis that the desired intestinal adaptive response following intestinal resection may be enhanced further by the administration of the locally active steroid budesonide, and by feeding a saturated as compared with a polyunsaturated fatty acid diet.

2) Methods and Materials

2.1) Western immunoblotting

BBM and BLM [illegible]quots of at least three animals in each group, containing roughly 20 mg of proteins, were solubilized in Sample Buffer Dye (0.125 M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 10% b-mercaptoethanol, 0.025% Bromophenol Blue). The Bromophenol Blue permits the visualization of the proteins. These samples were incubated at 100°C during 5 minutes to denature the proteins so that they will only migrate according to their molecular weight. Aliquots were stored at -20°C overnight.

BLM and BBM proteins were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) using a modification of the method developed by Laemmli (1970). Four gels were prepared in a multicaster chamber (Hoefer scientific instruments, San Francisco, California) and stored at 4°C overnight. Gels were composed of two parts: Resolving gel (7.5% gel, 0.375 M Tris, pH 8.8) and Stacking gel (4% gel, 0.123 M Tris, pH 6.8).

After rinsing the wells with deionized water, the samples were loaded using a syringe (Hamilton Company, Reno, Nevada). Each gel was completed with eighteen samples and two Kaleidoscope Prestained Standards (Bio-Rad laboratories, Hercules, Canada), as a consequence, 72 samples were ran per electrophoresis. Electrophoresis was carried out in a Hoefer electrophoresis tank (Hoefer scientific instruments, San Francisco, California). Gels were oriented vertically and submerged in a tank containing electrophoresis buffer (0.025 M Tris, pH 8.3, 0.192 M glycine, 0.1% SDS). Electrophoresis was ran at room temperature at a constant voltage of 100 Volts through the stacking gel for 30 minutes and 200 Volts through the resolving gel for 2 h 30.

After migration, proteins were immobilized on a solid support by electroblotting to a nitrocellulose membrane. Gel was put on contact with a nitrocellulose membrane and closed tightly in a transfer cassette (Hoefer scientific instruments, San Francisco, California) between filter papers and sponges. Cassettes were placed in a Hoefer transphor tank (Hoefer scientific instruments, San Francisco, California) between two electrode panels and totally submerged in Transfer Buffer (25mM Tris, 192mM glycine, 20% methanol) freshly prepared. Electrotransfer was carried out for 160 minutes at a current of 1 Ampere. In order to determine the completeness of protein transfer, the membranes were removed from the cassette and stained with Ponceau S (3-hydroxy-4-(2-sulfo-4-[4-sulfophenylazo]-phenylazo)-2, 7-naphthalenediasulfonic acid). Membranes were destained with deionized water until no further trace of Ponceau S was visible. Gels were stained with Coomassie Blue Stain (Coomassie Blue R250, methanol, deionized water and glacial acetic acid) to check if all proteins had been transferred from the gel to the nitrocellulose membrane. Then, the membranes were blocked by incubation overnight in BLOTTO (Bovine Lacto Transfer Technique Optimizer) containing 5% w/v dry milk in Tween Tris Buffered Saline (TTBS: 0.5% Tween 20, 30mM Tris, 150 mM NaCl). This step permits the saturation of non-specific protein binding sites to have a more specific binding with antibodies as well as a decrease in the background.

Membranes were washed three times with TTBS for at least 10 minutes each time and shook at setting of 2-3 on a shaker. Then, membranes were probed with specific rabbit anti-rat antibodies, that bound specifically to the antigens of interest. The incubation was carried out at room temperature, for 2 hours for GLUT2, GLUT5. The antibodies were diluted in 2% dry milk in TTBS at 1:500.

The polyclonal antibodies against GLUT2 were obtained from Biogenesis, Poole, England. They detect BLM proteins of 59 kDa. The polyclonal antibody against GLUT5 was obtained from Chemicon International Inc., Temecula, California. This antibody detects BBM proteins of 45 kDa.

Following this primary incubation, membranes were washed three times with TTBS to remove the residual unbound primary antibody. Membranes were then incubated during one hour with goat anti-rabbit antibody conjugated with horseradish peroxidase, HRP, (Pierce, Rockfort, Illinois, USA) that bind to the primary antibody. This second antibody was diluted at 1:20000 in 2% dry milk in TTBS.

After three washes in TTBS to remove residual secondary antibody, membranes were incubated 5 minutes with SuperSignal® Chemiluminescent-HRP Substrate (Pierce, Rockfort, Illinois, USA) composed of 50% Stable Peroxide Solution and 50% of Luminol/Enhancer Solution, that reacted with the secondary antibody and made visible the antigens of interest. Then, membranes were exposed to X-OMAT AR films for various times and successively plunged into developer, water and fixer.

Each membrane could be used twice with different antibodies provided that their molecular weights were not close. In this case, the membrane was stripped for 15 minutes and blocked overnight to get rid of the secondary antibody already bound. Then, the membrane was washed three times with TTBS and incubated with the other antibody.

The relative band densities were determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life science group, Cleveland, Ohio, USA). Samples that did not appear on the films or that were suspicious in accordance with the other samples of the films were repeated. Just one value per sample was kept for the capture of the data.

2.2) Northern Immunoblotting

For this experiment, complementary DNA (cDNA) probes were produced. Bacteria (*E. coli*) were transformed with plasmids containing the DNA sequences we wanted to use as a probe for the Northern blotting. cDNA probes encoding GLUT5 and GLUT2 were obtained from Dr. Bell, University of Chicago; ERG probes were obtained from Oncogene Research Products; cDNA probe encoding proglucagon was obtained form Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; and ODC was obtained from Dr. Blackshear, University of Chicago. They were grown on agar plates, transferred to LB broth and the plasmid DNA was isolated. To make the cDNA probe, the DNA insert in the plasmid was cut by 2 specific restriction enzymes (Gibco BRL, Life Technologies, USA). A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labelled DNA (Roche Diagnostics, Quebec, CA) that was dotted on the same membrane.

RNA was extracted from the jejunum and ileum of at least three animals in each group. These segments were homogenized in a denaturing solution, containing guanidinium thiocyanate, using the biorad fast prep shaking centrifuge (BIO101, Vista, California, USA). Following addition with 2M sodium acetate, a phenol chloroform extraction was done. The upper aqueous phase was transferred to a tube and the RNA was precipitated with isopropanol and washed with 70% ethanol.

Equal amount of total RNA were denatured in a sample loading buffer. Ethidium bromide (10 mg/ml) was added so that the integrity of the RNA can be determined by visualizing the 28S and 18S ribosomal bands under UV light.

Total RNA was separated based on molecular weight as it was electrophoresed through a denaturing agarose gel (1.16% agarose). The migration lasted 5 hours at a current level of 100 volts in an electrophoresis tank (HLB12 Complete Horizontal Long Bed Gel System, Tyler, Edmonton, CA). RNA was then transferred form the gel to a nylon membrane by capillary action, overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA onto it.

As a pre-hybridization, membranes were incubated during 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at 42°C overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid) in order to reduce non-specific binding sites. They were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was done using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA) and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA). To determine the exact RNA quantity that has been loaded, the 28S ribosomal band density on the membrane was evaluated after the transfer. At the end of the experiment, the RNA band density of the film was evaluated. The ratio between the RNA band density value onto the film and onto the membrane was calculated.

3) Results

3.1) Animal Characteristics

In transected rats, food intake was higher in the animals fed chow rather than with the SFA or PUFA (Table 69). Body weight gain and weight gain per food intake were lower with SFA than with PUFA or chow. In resected animals fed PUFA, both weight gain and weight gain per food intake were lower than in transected animals fed PUFA, but were similar in resected rats fed PUFA as compared with SFA or chow.

Dexamethasone reduced body weight gain in resected animals fed chow, SFA or PUFA (Table 70). Only in PUFA was this associated with reduced food intake. In contrast, budesonide and prednisone had no effect on food intake or body weight gain in resected rats fed chow, SFA or PUFA. Resected rats given budesonide or prednisone gained more weight when fed PUFA, as compared with those fed SFA.

In transected rats, the total weight of the intestine and the percentage of the intestinal wall comprised of scrapable mucosa was similar in the jejunum and in the ileum of rats fed chow, SFA or PUFA (Table 71). The weight of the jejunum was greater in resected than in transected rats fed PUFA, but the percentage of the intestinal wall comprised of mucosa was less (23.9 ± 1.9 vs 18.4 ± 21.2 and 45.9 ± 2.3 vs 57 ± 5.2 respectively). There was no difference in the intestinal characteristics of resected versus transected animals fed chow or SFA.

Giving budesonide, prednisone or dexamethasone to rats fed chow, SFA or PUFA had no effect on the weight per centimeter length of intestine remaining after resection, or on the percentage of the intestinal wall comprised of mucosa (Table 72). The weight of the jejunal wall was approximately one-third lower in resected rats given budesonide or prednisone and fed PUFA, as compared with those fed SFA or chow, and the percentage of the intestinal wall comprised of mucosa was increased in animals given dexamethasone and fed PUFA.

3.2) **Sugar Uptake**

There was a curvilinear relationship between glucose concentration and uptake in all groups, except for ileal uptake of glucose in transected rats fed SFA (data not shown). Variations in the lipid content of the diet had no effect on the value of the maximal transport rate (Vmax) of the jejunal or ileal uptake of glucose in transected or resected animals (Table 73). The value of the apparent Michaelis constant (Km) was lower in the jejunum of resected as compared with transected rats fed PUFA, but resection or dietary lipid alterations had no effect on the value of the Km in the other groups.

In the jejunum of resected rats fed chow, budesonide increased the values of Vmax and Km as compared to those given control vehicle, prednisone or dexamethasone (Table 74). The value of the Vmax was lower in rats given prednisone and fed SFA.

None of the steroids had an effect on the values of Vmax or Km for the ileal uptake of glucose in animals fed chow, SFA or PUFA. Thus, budesonide increases glucose uptake, rather than causing a decline in glucose uptake as is seen with prednisone. Thus, feeding PUFA rather than SFA prevents the increased jejunal glucose uptake (increase value of the Vmax) in rats given budesonide.

The rate of jejunal uptake of L-glucose was lower in transected rats fed PUFA as compared with SFA or chow (Table 75). In contrast, the jejunal uptake of L-glucose was higher in resected than in transected animals fed PUFA. The ileal uptake of L-glucose was not affected by resection or by dietary lipids. The three steroids had no effect on L-glucose uptake into the jejunum of rats fed chow, SFA or PUFA (Table 76). In the ileum of resected animals fed SFA, prednisone reduced L-glucose uptake as compared with those given budesonide, dexamethasone or control vehicle.

There was a linear relationship between fructose concentration and uptake (data not shown). For this reason it was not possible to calculate the values for Vmax or Km. Accordingly, the influence of surgery, diet and steroids on fructose uptake is reported as an effect on the slope of this linear relationship between fructose concentration and uptake. There were no differences in the rates of uptake of D-fructose into the jejunum or ileum of resected versus transected rats fed chow, SFA or PUFA (Table 77). In transected and in resected animals, fructose uptake into the jejunum was lower in PUFA than in SFA or chow. Ileal uptake of D-fructose was also lower in transected rats fed PUFA versus SFA or chow.

In rats fed chow, dexamethasone reduced fructose uptake as compared with those given prednisone, budesonide or control vehicle (Table 78). Budesonide reduced jejunal uptake of D-fructose in resected rats fed SFA but not PUFA. Prednisone did not affect fructose uptake. Feeding SFA or PUFA and giving budesonide reduced fructose uptake as compared with rats fed chow in either jejunum and ileum. Feeding PUFA reduced the uptake of fructose, as compared with rats fed SFA. In the ileum, budesonide increased fructose uptake in resected rats fed chow or PUFA, but not in those fed SFA. Ileal uptake of fructose was lower with budesonide- or prednisone-treated rats fed SFA or PUFA as compared with chow. Feeding PUFA as compared with SFA reduces the jejunal uptake of fructose observed in rats given control vehicle, dexamethasone or prednisone.

3. Transporter Protein Abundance and Expression of mRNA

SGLT1 abundance was decreased in the ileum of resected animals given control and fed SFA or PUFA as compared to those fed chow (Table 79). Na^+/K^+ ATPase alpha 1 expression was decreased in the jejunum of resected animals given control and fed PUFA as compared to those fed SFA or chow. Na^+/K^+ ATPase beta 1 was decreased in the ileum of animals undergone intestinal resection, given budesonide and fed PUFA as compared to those fed chow. SGLT1 and Na^+/K^+ ATPase alpha 1 mRNAs were unchanged in either jejunum and ileum whether the animals were fed chow, SFA or PUFA (Table 80). However, mRNA for Na^+/K^+ ATPase beta 1 was decreased in the ileum of resected animals given control and fed PUFA as compared to those fed SFA and chow.

Steroids did not affect the expression of SGLT1, but budesonide, prednisone and dexamethasone in animals fed PUFA reduced the ileal abundance of Na^+/K^+ ATPase alpha 1 as compared to control vehicle (Table 81). Budesonide and dexamethasone increased the ileal Na^+/K^+ ATPase beta 1 expression as compared to control and prednisone in resected animals fed chow. Prednisone increased the jejunal abundance of Na^+/K^+ ATPase alpha 1 mRNA as compared to budesonide in those fed SFA, and prednisone and dexamethasone decreased the ileal expression of Na^+/K^+ ATPase beta 1 as compared to control, in those fed SFA (Table 82). SGLT1 mRNA expression was unchanged by the administration of steroids.

SGLT1 and Na^+/K^+ ATPase alpha 1 and beta 1 expression in the jejunum was not altered by resection (Table 83). However, in the ileum, the expression of SGLT1 was increased by resection whereas the expression of Na^+/K^+ ATPase alpha 1 was reduced, and the expression of Na^+/K^+ ATPase beta 1 was unchanged. Resection did not change the mRNA expression for SGLT1, Na^+/K^+ ATPase alfa 1 and beta 1 in the ileum, but reduced the jejunal expression of both Na^+/K^+ ATPase subunits (Table 84).

GLUT5 and GLUT2 abundances were not altered in the jejunum by variations in the diet (Table 85). In the ileum, GLUT5 abundance was decreased in resected rats fed SFA or PUFA and given budesonide as compared to those fed chow. This reduction in GLUT5 expression was bigger in those fed SFA. Ileal GLUT2 expression was also decreased in resected animals fed SFA or PUFA and given dexamethasone, as compared to those fed chow. mRNA for GLUT5 was reduced by SFA and PUFA diets as compared to chow in the jejunum of resected rats given prednisone (Table 86), and it was again reduced by SFA diet as compared to PUFA and chow diets in the ileum of resected animals given prednisone. GLUT2 mRNA did not change in the jejunum, but it was decreased by PUFA as compared to chow in the ileum of animals given prednisone.

GLUT5 and GLUT2 expression in resected rats was not altered by the administration of budesonide, prednisone or dexamethasone (Table 87). The messenge for these transporters was also unchanged in the ileum of resected animals (Table 88). However, in the jejunum, GLUT5 mRNA was increased by prednisone as compared to control vehicle, prednisone or dexamethasone in those fed chow or SFA.

Resection did not have any effect on the abundance of GLUT5 and GLUT2 (Table 89). GLUT5 mRNA was reduced in the jejunum of resected animals as compared to transected animals, and unchanged in the ileum (Table 90). GLUT2 mRNA was unchanged whether the animals were resected or transected.

3.4) Early Response Genes

Dietary lipids did not alter the expression of the mRNAs for the early response gene c-myc, but SFA and PUFA increased the expression of c-jun in the jejunum of resected rats given budesonide as compared to those fed chow (1.16 ± 0.02 and 1.06 ± 0.04 vs 1 ± 0; Table 91). No differences in the expression of c-jun were observed in the ileum. The administration of steroids did not modify the expression of c-fos and c-jun in resected rats fed SFA or PUFA (Table 92). In chow fed animals, all steroids reduced the jejunal c-jun expression. Intestinal resection did not alter the expression c-myc and c-jun as compared to animals undergoing intestinal transection (Table 93).

3.5) Proglucagon and ODC mRNA Expression

Animals undergoing intestinal resection, fed SFA and given control vehicle had a reduction in jejunal proglucagon mRNA expression as compared to those fed chow or PUFA (Table 94). Resected animals fed SFA and given budesonide had a reduction in jejunal ODC mRNA expression, as compared to those fed chow or PUFA. In the ileum, ODC mRNA expression was unaffected by steroids. Ileal proglucagon mRNA expression was increased in animals fed PUFA and given prednisone as compared to those fed SFA or chow.

Steroids reduced the jejunal mRNA expression of proglucagon in resected animals fed chow, but did not change ileal expression (Table 95). Prednisone given to rats fed SFA increased the jejunal mRNA expression of proglucagon. ODC mRNA was not affected by steroids in rats fed chow, SFA or PUFA.

Proglucagon mRNA expression in the jejunum and ileum was not changed by resection (Table 96) . ODC mRNA expression of resected animals was reduced in the jejunum (0.52 ± 0.12 vs 1 ± 0) but not in the ileum.

4) Discussion

The intestinal absorption of nutrients is subject to adaptation of both mediated- and non-mediated processes, with alteration of transport influenced by the animal's age, composition of the diet, as well as by pathological processes such as diabetes mellitus, chronic ethanol intake and abdominal irradiation [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, morphological and functional changes occur depending upon the extent of the intestine removed, the site studied, and the lipid content of the diet [Keelan et al., 1996]. The signal for this process is unknown, but may include proglucagon-derived peptides, ERGs and ODC [Bloom and Polak, 1982; Rountree et al., 1992; Tappenden et al., 1996]. ERGs such as c-myc, c-jun and c-fos have been demonstrated to be involved in processes of proliferation and differentiation, as well as ODC, a key enzyme in the synthesis of polyamines and a requirement in any proliferative event [Bloom and Polak, 1982; Rountree et al., 1992; Tappenden et al., 1996; Sagor et al., 1983]. Proglucagon contributes to the intestinal adaptive process [Mosjov et al., 1986; Orskov et al., 1987]. For example, short chain fatty acids increases the ileal c-myc and proglucagon expression in rats undergoing intestinal resection [Tappenden et al., 1996]. In this study, we have shown involvement of c-jun, proglucagon and ODC in the adaptive response. For instance, animals fed SFA and given budesonide increased their jejunal expression of c-jun as compared to those fed PUFA or chow and given budesonide, steroids reduced the jejunal expression of c-jun in animals fed chow, animals fed SFA and given budesonide had a reduction in jejunal ODC expression as compared to those fed chow or PUFA, prednisone given to rats fed SFA increased their jejunal expression of proglucagon but steroids given to rats fed chow reduced the jejunal expression of proglucagon, and intestinal resection reduced the jejunal expression of ODC. It is not known the mechanisms by which these signals might alter the phenotypic alterations observed in transport activity. Of course, other signals may be involved in this adaptive response. Epimorphin/syntaxin 2 mRNA that codes for a membrane-associated protein involved in morphogenesis of the lungs and skin, and PC4/TIS7, a gene involved in nerve growth factor-mediated cytodifferentiation, may be among other signals that might be involved in the adaptive response after intestinal resection [Goyal et al., 1998; Rubin et al., 1998]. New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001].

Glucocorticosteroids are used to treat patients with a variety of intestinal conditions, including Crohn's disease and ulcerative colitis. Recent clinical attention has focussed on the use of potent locally acting steroids such as budesonide [Campieri et al., 1995; Greenberg et al., 1994; Lofberg et al., 1993; Thiesen and Thomson, 1996; Thiesen et al. 1996; Ozcay et al., 1997]. Prednisone and budesonide modify the morphology and absorptive function of the intestine in young rats with an intact intestinal tract [Thiesen and Thomson, 1996]. This study extends these observat~~ions~~ to compare the influence of the locally active budesonide versus two systemically active steroids (prednisone given by mouth, and dexamethasone given by subcutaneous injection) in animals in which a portion of the jejunum and ileum remained after removal of the middle half of the small intestine.

The systemic administration of dexamethasone blunts the morphological adaptation of the intestine of adult rats when studied one week after an 80% enterectomy There is a drop in the weight, protein and DNA content of the intestine, as well as inhibition of the normal mucosa growth and mucosal hyperplasia [Park et al., 1994]. We used young rats studied two weeks after removal of the middle half of the small intestine (50% enterectomy). The extent of resection is known to result in morphological and functional adaptation [Keelan et al., 1996], and a 50% enterectomy rather than a massive 80% resection was chosen because sufficient proximal and distal intestine remained to assess the morphology and function at both sites. Furthermore, a 50% resection does not usually result in body weight loss [Keelan et al., 1996], and is closer to the clinical situation seen, for example, in some patients with Crohn's disease. In this study, food intake and body weight gain were unaffected by this 50% intestinal resection in rats fed chow or SFA (Table 69). Food intake was similar in transected and resected rats fed PUFA, but weight gain was lower in resected than in transected animals. The explanation for this lower weight gain is unclear, since the value of the Vmax for D-glucose uptake was unchanged (Table 73), the value of the D-fructose uptake was unchanged (Table 77), and the non-mediated uptake of L-glucose was not altered in rats fed chow (Table 75).

Although we used the same dose of dexamethasone which has been reported previously to blunt the expected morphological response to intestinal resection [Park et al., 1994], we were unable to demonstrate any adverse effect on the weight of the jejunum or ileum of resected animals. In fact, the percentage of the intestinal wall comprised of mucosa, and therefore the mucosal weights, were actually increased in the jejunum of resected animals fed PUFA. This lack of detrimental effect of dexamethasone on the intestine of resected animals may have been due to differences in animal strain, age, length of intestine resected, or time after resection when our studies were performed.

Neither budesonide nor prednisone altered the weight of the intestine, or the percentage of the intestinal wall comprised of mucosa in resected animals. It is possible that had we subjected the animals to a massive bowel resection (eg, 80% of the small intestine), then the weight of the remaining intestine per unit length or surface area might have changed. Nonetheless, based on this data two weeks following a 50% enterectomy, it is clear that these steroids given in these doses and by these routes have no adverse effects on the weight of the intestine. A similar lack of effect of budesonide and prednisone on intestinal weight has been reported in non-operated intestine [Thiesen et al., 1996]. Furthermore, these data underline the point that the effects of steroids on the absorptive functions of the intestine, to be discussed below, are not due to any steroid-induced change in the weight of the intestine.

While the absorption of nutrients may be increased after intestinal resection, the magnitude of this effect depends on the extent of resection, as well as on the site and time after surgery when experiments are performed [Hanson et al., 1977; Williamson et al., 1978; Williamson et al., 1982a; Williamson et al., 1982b]. We could have subjected the animals to a massive small bowel resection, and potentially been able to show enhanced uptake of sugars. However, the interpretation of the results then would have been influenced and been made difficult by the expected concomitant changes in food intake, body weight gain and intestinal weight. Furthermore, we wished these studies to have some potential clinical relevance. For example, in patients with Crohn's disease, prednisone or budesonide may be used therapeutically in the doses used here, and while these individuals previously may have undergone an intestinal resection, massive resections would be unusual.

There is a difference in the potency of these steroids on the absorption of sugars by the intestine after a 50% enterectomy: in animals fed chow, prednisone had no influence on the value of the Vmax of glucose uptake (Table 74) or on the uptake of fructose (Table 78), whereas budesonide increased the value of the Vmax for uptake of D-glucose (Table 74). As well, budesonide increased the ileal uptake of fructose in animals fed chow or PUFA (Table 78). This occurred without any change in the passive non-mediated component of sugar uptake, as measured with L-glucose (Table 76). The doses of prednisone and budesonide were chosen on the basis of regimens which have been shown to be useful clinically [Boyd et al., 1995; Campieri et al., 1995], and which have been studied previously in rats with an intact intestine [Thiesen et al., 1996]. These doses are similar to those used to treat trinitrobenzene sulphonic acid ileitis in rats [Boyd et al., 1995], and to the doses used to prevent graft rejection in a rat model of intestinal transplantation [Ozcay et al., 1997].

Protein and mRNA expression of the transporters responsible for the uptake of glucose and fructose, SGLT1 and GLUT5 respectively, did not correlate with the activity of these transporters as assessed for the rates of uptake of these sugars. For instance, the enhancement in glucose uptake with budesonide in the jejunum (Table 74) was not accompanied by an increase in the expression of SGLT1 (Table 81). Furthermore, the mRNA expression for SGLT1 was also unchanged (table 82). These findings suggest a post-translational regulation of SGLT1. The same was observed for GLUT5. The enhanced fructose uptake with budesonide in the ileum (Table 78) was not accompanied by changes in GLUT5 and mRNA expression (Tables 87 and 88), suggesting again a post-translational event involved in the regulation of fructose uptake. These findings suggest the possibility of post-translational regulation of GLUT5 and SGLT1. We did not perform immunohistochemistry of the distribution of these transporters along the villus so that it is possible that the changes in uptake were associated with alterations in the distribution of transporters along the intestinal villus, without any changes being measured by Western blotting in the total abundance of the transporter. Recent evidence that GLUT2 may be also located on the BBM [Helliwell et al., 2000; Helliwell et al., 2000; Kellet, 2001], suggests the possibility that the abundance of this transporter in this new location may change under the experimental conditions presented in this study.

The systemic bioavailability of budesonide is approximately one order of magnitude lower than for prednisone, its potency is higher [Greenberg et al., 1994; Lofberg et al., 1993; Rutgeerts et al., 1994; Thiesen and Thomson, 1996]. We suggest that the stimulating effect of budesonide on the intestinal uptake of glucose in animals fed chow, and its stimulating effect on the uptake of fructose in rats fed chow and PUFA, is the result of its greater effect on the receptors for steroids in the enterocytes.

Isocaloric changes in dietary lipids modify the intestinal absorption of nutrients in adult rats, with greater uptake of sugars, amino acids and fatty acids in animals fed a semisynthetic SFA versus PUFA diet [Thomson, 1982; Thomson et al., 1991]. This observation has been applied to purposely modify the adaptation of the intestine in a manner which achieves a therapeutic objective. For example, feeding SFA enhances uptake and prevents or reduces the malabsorption which otherwise would occur following the chronic intake of ethanol [Thomson et al., 1991], or following abdominal irradiation [Thomson et la., 1989]. Furthermore, the undesired enhanced uptake of sugars and lipids which occurs in experimental diabetes mellitus may be prevented by feeding PUFA [Thomson et la., 1987]. This has the additional clinical advantage of improving glucose control, and reducing the hyperlipidemia and elevated concentrations of hemoglobin A1c [Keelan et al., 1989; Rajotte et al., 1988; Thomson et al., 1988]. Indeed, the desired enhancement of nutrient transport in rats following a 50% intestinal resection requires the presence of SFA in the diet [Keelan et al., 1985].

Only in resected rats fed SFA was prednisone associated with a reduced Vmax for glucose (Table 74), and prednisone was associated with reduced absorption of fructose in resected rats fed PUFA as compared with those fed chow or SFA (Table 78). This malabsorption of sugars with prednisone was of potential nutritional significance, since it was associated with lower body weight gain (Table 70).

Steroids may increase fructose uptake in animals with an intestinal resection, but this beneficial effect is prevented in rats fed SFA rather than PUFA. It is possible that in the setting of bowel resection, budesonide might be advantageous as compared with prednisone, with greater ileal uptake of fructose (Table 78), and with no unwanted loss of body weight. It remains to be determined whether budesonide may play a therapeutic role to stimulate intestinal adaptation following intestinal resection in humans.

The results demonstrate that budesonide increases the uptake of fructose in rats fed SFA, whereas prednisone and dexamethasone reduces fructose uptake in PUFA versus SFA. The influence of steroids on the transport function of the intestine is modified by the lipid content of the diet; ileal uptake of fructose is up-regulated by the locally acting budesonide; and the loss of body weight observed in animals given dexamethasone can be prevented by giving either budesonide or prednisone. It remains to be determined whether budesonide may play a therapeutic role to stimulate intestinal adaption following intestinal resection in humans.

Table 69. Effect of Diet and Resection on Food Consumption and Body Weight Gain

Diet	Food Intake (g/day)	Body Weight Gain (g/day)	Weight Gain per Food Intake(%)
Transected			
Chow	23.0 ± 1.2	6.3 ± 0.3	27.8 ± 1.0
Saturated	17.8 ± 0.4 &	4.2 ± 0.2 &	23.4 ± 1.0 &
Polyunsaturated	18.8 ± 0.6 &	6.5 ± 0.2 #	34.7 ± 0.9 &#
Resected			
Chow	20.7 ± 1.3	4.8 ± 0.9	30.5 ± 4.1
Saturated	17.4 ± 0.4	3.2 ± 0.6	18.2 ± 2.9
Polyunsaturated	18.0 ± 0.5	5.1 ± 0.4 +	28.1 ± 1.9 +

Mean ± SEM

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

+, $p<0.05$, transection vs resection

Table 70. Effect of Steroids on Food Consumption and Body Weight Gain in Rats Undergoing Intestinal Resection

Diet	Drug	Food Intake (g/day)	Body Weight Gain (g/day)	Weight Gain per Food Intake (%)
Chow	Control	20.7 ± 1.3	4.8 ± 0.9	30.5 ± 4.1
	Budesonide	20.0 ± 2.9	3.6 ± 0.5	18.1 ± 1.3 *
	Prednisone	20.2 ± 0.4	4.4 ± 0.4	24.5 ± 1.8 ~
	Dexamethasone	25.5 ± 2.7	2.0 ± 0.3 *	6.9 ± 0.8 *~^
Saturated	Control	17.4 ± 0.4 &	3.2 ± 0.6	18.2 ± 2.9 &
	Budesonide	18.0 ± 0.3	3.2 ± 0.5	17.8 ± 2.4
	Prednisone	17.3 ± 0.2 &	2.9 ± 0.3 &	17.1 ± 1.5 &
	Dexamethasone	17.0 ± 0.3 &	-0.2 ± 0.1 *~^&	-1.1 ± 0.8 *~^&
Polyunsaturated	Control	18.0 ± 0.5 &	5.1 ± 0.4	28.1 ± 1.9 #
	Budesonide	17.4 ± 0.8	5.1 ± 0.6 #	29.4 ± 3.2 &#
	Prednisone	16.3 ± 0.2 #&	4.9 ± 0.2 #	30.1 ± 1.2 &#
	Dexamethasone	15.5 ± 0.6 *&	1.3 ± 0.3 *~^&	8.3 ± 2.1 *~^#

Mean ± SEM

*, p<0.05, budesonide, prednisone or dexamethasone vs control

~, p<0.05, prednisone or dexamethasone vs budesonide

^, p<0.05, dexamethasone vs prednisone

#, p<0.05, polyunsaturated vs saturated fatty acid diet

&, p<0.05, saturated or polyunsaturated fatty acid diet vs chow diet

Table 71. Effect of Diet and Resection on Characteristics of Intestine

Diet		Intestinal Weight (mg/cm)	Intestinal Wall Comprised of Mucosa (%)
Transected			
Chow	jejunum	19.5 ± 3.0	51.6 ± 1.9
	ileum	15.0 ± 2.5	52.7 ± 4.7
Saturated	jejunum	22.3 ± 2.3	61.0 ± 6.3
	ileum	10.3 ± 2.3	61.0 ± 6.3
Polyunsaturated	jejunum	18.4 ± 1.2	57.0 ± 5.2
	ileum	10.9 ± 1.5	54.4 ± 7.0
Resected			
Chow	jejunum	23.4 ± 3.7	47.5 ± 4.1
	ileum	17.5 ± 4.8	46.4 ± 4.7
Saturated	jejunum	22.8 ± 2.5	52.1 ± 2.2
	ileum	14.3 ± 2.4	51.5 ± 5.6
Polyunsaturated	jejunum	23.9 ± 1.9 +	45.9 ± 2.3 +
	ileum	12.8 ± 2.1	50.4 ± 1.9

Mean ± SEM

+, $p<0.05$, transection vs resection

Table 72. Effect of Steroids on the Characteristics of Intestine of Rats Undergoing Intestinal Resection

Diet		Drug	Intestinal Weight (mg/cm)	Intestinal Wall Comprised of Mucosa (%)
Chow	Jejunum	Control	23.4 ± 3.7	47.5 ± 4.1
		Budesonide	23.1 ± 2.3	48.7 ± 4.1
		Prednisone	24.2 ± 1.7	48.5 ± 7.2
		Dexamethasone	21.1 ± 2.5	64.2 ± 2.4
	Ileum	Control	17.5 ± 4.8	46.4 ± 4.7
		Budesonide	14.5 ± 0.7	40.4 ± 6.0
		Prednisone	13.5 ± 1.7	52.2 ± 2.0
		Dexamethasone	9.4 ± 1.8	52.2 ± 2.1
Saturated	Jejunum	Control	22.8 ± 2.5	52.1 ± 2.2
		Budesonide	24.4 ± 1.8	5.29 ± 2.9
		Prednisone	28.3 ± 1.8	54.4 ± 6.6
		Dexamethasone	21.3 ± 1.4	56.1 ± 5.8
	Ileum	Control	14.3 ± 2.4	51.5 ± 5.6
		Budesonide	15.7 ± 2.3	45.0 ± 2.8
		Prednisone	14.3 ± 0.8	48.1 ± 5.6
		Dexamethasone	10.3 ± 1.3	46.3 ± 5.9
Polyunsaturated	Jejunum	Control	23.9 ± 1.9	45.9 ± 2.3
		Budesonide	16.6 ± 1.3 #&	54.0 ± 3.0
		Prednisone	16.3 ± 2.4 #&	53.1 ± 6.0
		Dexamethasone	20.2 ± 2.1	65.6 ± 3.1 *
	Ileum	Control	12.8 ± 2.1	50.4 ± 1.9
		Budesonide	12.5 ± 3.7	44.5 ± 6.0
		Prednisone	13.4 ± 1.9	56.3 ± 4.1
		Dexamethasone	9.7 ± 1.8	56.5 ± 4.9

Mean ± SEM

*, p<0.05, budesonide, prednisone or dexamethasone vs control

#, p<0.05, polyunsaturated vs saturated fatty acid diet

&, p<0.05, saturated or polyunsaturated fatty acid diet vs chow diet

Table 73. Effect of Diet and Resection on the Values of the Maximal Transport Rate (Vmax) and Michaelis Affinity Constant (Km) of D-Glucose Uptake

	Jejunum		Ileum	
Diet	Vmax	Km	Vmax	Km
Transected				
Chow	2986 ± 392	40.8 ± 10.3	5083 ± 1043	134 ± 37.4
Saturated	11310 ± 4780	318.4 ± 156.6	@	@
Polyunsaturated	4221 ± 478	118.7 ± 18.8	6394 ± 2558	262.7 ± 125.7
Resected				
Chow	3066 ± 217	39.7 ± 5.5	2726 ± 320	59.7 ± 11.9
Saturated	3503 ± 202	36.1 ± 4.2	4124 ± 627	81.7 ± 19.2
Polyunsaturated	3861 ± 665	57.8 ± 17.1 +	4564 ± 1065	151.1 ± 46.7

Mean ± SEM

Vmax has the units nmol*100 mg mucosal tissue^{-1}*min^{-1}, and Km has the units mM.

+, p<0.05, transection vs resection

@, unable to calculate kinetic constants (Vmax and Km) because of a linear relationship between concentration and uptake

Table 74. Effect of Steroids on the Values of the Maximal Transport Rate (Vmax) and Michaelis Affinity Constant (Km) in the Jejunum and Ileum of Rats Undergoing Intestinal Resection

		Jejunum		Ileum	
Diet	Drug	Vmax	Km	Vmax	Km
Chow	Control	3066 ± 217	39.7 ± 5.5	2726 ± 320	59.7 ± 11.9
	Budesonide	6790 ± 803 *	93.7 ± 16.5 *	5091 ± 964	131.1 ± 34.0
	Prednisone	2531 ± 535 ~	35.7 ± 15.2 ~	9354 ± 3313	239.3 ± 102.8
	Dexamethasone	2991 ± 283 ~	39.3 ± 7.3 ~	5062 ± 2464	118.3 ± 80.7
Saturated	Control	3503 ± 202	36.1 ± 4.2	4124 ± 627	81.7 ± 19.2
	Budesonide	3571 ± 147	45.4 ± 3.5	6167 ± 1864	134.9 ± 55.4
	Prednisone	2268 ± 250*~	35.2 ± 7.9	5903 ± 1353	151.5 ± 46.0
	Dexamethasone	3566 ± 278^	47.8 ± 6.8	6411 ± 874	110.2 ± 21.4
Polyunsaturated	Control	3861 ± 665	57.8 ± 17.1	4564 ± 1065	151.1 ± 46.7
	Budesonide	4359 ± 1191	88.8 ± 36.7	7588 ± 1482	176.9 ± 44.3
	Prednisone	3840 ± 685	62 ± 18.6	5149 ± 1467	198 ± 70.7
	Dexamethasone	2433 ± 155 #	45.7 ± 5.4	5802 ± 364	99.4 ± 9.2

Mean ± SEM

Vmax has the units nmol*100 mg mucosal tissue^{-1}*min^{-1}, and Km has the units mM.

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

Table 75. Effects of Diet and Resection on Uptake of L-Glucose

Diet	Jejunum	Ileum
Transected		
Chow	25.9 ± 2.2	26.4 ± 2.2
Saturated	26.5 ± 2.2	31.1 ± 2.7
Polyunsaturated	15.8 ± 1.2 &#	24.3 ± 2.6
Resected		
Chow	24.1 ± 1.6	23.6 ± 2.9
Saturated	22.2 ± 1.3	29.6 ± 2.7
Polyunsaturated	24.4 ± 1.5 +	20.5 ± 1.3

Mean ± SEM

The rate of uptake of L-glucose has the units nmol*100 mg mucosal tissue^{-1}*min^{-1}*mM^{-1}.

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

+, $p<0.05$, transection vs resection

Table 76. Effects of Steroids on Uptake of L-Glucose in Resected Rats

Diet	Drug	Jejunum	Ileum
Chow	Control	24.1 ± 1.6	23.6 ± 2.9
	Budesonide	27.7 ± 2.4	24.2 ± 2.9
	Prednisone	25.8 ± 2.5	27.0 ± 2.7
	Dexamethasone	20.0 ± 1.4	25.0 ± 2.4
Saturated	Control	22.2 ± 1.3	29.6 ± 2.7
	Budesonide	23.8 ± 2.0	28.8 ± 3.2
	Prednisone	22.1 ± 2.6	18.0 ± 1.8 *~
	Dexamethasone	25.1 ± 2.7	31.1 ± 3.4 ^
Polyunsaturated	Control	24.4 ± 1.5	20.5 ± 1.3 #
	Budesonide	24.4 ± 1.6	26.8 ± 2.4
	Prednisone	29.0 ± 2.9	23.6 ± 3.0
	Dexamethasone	20.8 ± 1.5	27.0 ± 2.8

Mean ± SEM

The rate of uptake of L-glucose has the units nmol*100 mg mucosal tissue^{-1}*min^{-1}*mM^{-1}.

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

Table 77. Effect of Diet on Uptake of D-Fructose

Diet	Jejunum	Ileum
Transection		
Chow	34.4 ± 1.9	26.6 ± 2.0
Saturated	28.8 ± 1.2 &	25.8 ± 1.9
Polyunsaturated	22.6 ± 1.3 &#	17.1 ± 1.3 &#
Resection		
Chow	31.9 ± 1.5	26.5 ± 2.8
Saturated	33.6 ± 2.2	24.1 ± 1.6
Polyunsaturated	26.2 ± 1.4 &#	17.5 ± 0.8

Mean ± SEM

The uptake of fructose has the values nmol*100 mg mucosal tissue^{-1}*min^{-1}.

#, p<0.05, polyunsaturated vs saturated fatty acid diet

&, p<0.05, saturated or polyunsaturated fatty acid diet vs chow diet

Table 78. Effect of Steroids on Uptake of D-Fructose in Rats Undergoing Intestinal Resection

Diet	Drug	Jejunum	Ileum
Chow	Control	31.9 ± 1.5	26.5 ± 2.8
	Budesonide	30.4 ± 2.3	45.0 ± 3.8 *
	Prednisone	33.5 ± 2.3	27.3 ± 1.9 ~
	Dexamethasone	23.3 ± 1.4* ~^	23.2 ± 1.5 ~
Saturated	Control	33.6 ± 2.2	24.1 ± 1.6
	Budesonide	24.7 ± 1.5 *&	19.7 ± 1.9 &
	Prednisone	31.8 ± 1.9 ~	19.7 ± 1.8 &
	Dexamethasone	28.8 ± 1.6 &	27.5 ± 2.1 ~^
Polyunsaturated	Control	26.2 ± 1.4 &#	17.5 ± 0.8 &#
	Budesonide	23.2 ± 1.7 &	25.7 ± 1.8 *&
	Prednisone	24.1 ± 2.0 &#	21.3 ± 2.2 &
	Dexamethasone	20.8 ± 1.4 #	27.7 ± 2.0 *^

Mean ± SEM

The uptake of fructose has the units nmol*100 mg mucosal tissue^{-1}*min^{-1}.

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 79. Effect of Diets on the Expression of SGLT1, Na^+/K^+ ATPase Alpha 1 and Beta1

Drug		Diet	SGLT1	Alpha 1	Beta 1
Control	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.35 ± 1.15	1.03 ± 0.26	0.83 ± 0.59
		Polyunsaturated	1.08 ± 0.36	0.14 ± 0.19&#	1.03 ± 1.05
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.49 ± 0.20&	4.64 ± 3.86	2.84 ± 3.12
		Polyunsaturated	0.38 ± 0.30&	2.25 ± 2.69	9.58 ± 16.02
Budesonide	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	7.26 ± 12.02	1.25 ± 0.71	0.76 ± 0.34
		Polyunsaturated	4.92 ± 7.03	0.40 ± 0.36	1.41 ± 1.36
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.23 ± 0.13	0.52 ± 0.48	0.65 ± 0.19
		Polyunsaturated	1.01 ± 0.87	1.01 ± 1.63	0.38 ± 0.25&
Prednisone	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.56 ± 0.62	7.17 ± 9.42	0.60 ± 0.11
		Polyunsaturated	2.46 ± 2.37	8.35 ± 13.52	0.95 ± 0.82
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.71 ± 0.44	5.35 ± 7.62	0.70 ± 0.39
		Polyunsaturated	0.85 ± 0.34	0.91 ± 1.08	0.60 ± 0.59
Dexamethasone	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.87 ± 0.43	2.93 ± 2.94	1.30 ± 0.74
		Polyunsaturated	0.53 ± 0.20	2.11 ± 2.03	1.02 ± 0.40
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.21 ± 0.64	1.25 ± 1.23	0.45 ± 0.30
		Polyunsaturated	1.09 ± 0.59	0.26 ± 0.37	0.42 ± 0.40

Mean ± SD

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 80. Effect of Diets on the mRNA for SGLT1, Na^+/K^+ ATPase Alpha 1 and Beta1

Drug		Diet	SGLT1	Alpha 1	Beta 1
Control	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.78 ± 0.54	1.09 ± 0.43	1.12 ± 0.51
		Polyunsaturated	1.13 ± 0.70	1.34 ± 0.66	1.54 ± 0.96
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.18 ± 0.89	1.02 ± 0.61	0.92 ± 0.15
		Polyunsaturated	0.85 ± 0.25	0.79 ± 0.10	0.65 ± 0.21&#
Budesonide	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.97 ± 0.11	0.82 ± 0.24	1.01 ± 0.35
		Polyunsaturated	1.06 ± 0.29	0.86 ± 0.13	1.01 ± 0.24
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.93 ± 0.42	1.00 ± 0.21	0.87 ± 0.22
		Polyunsaturated	1.19 ± 0.45	1.63 ± 0.67	1.23 ± 0.53
Prednisone	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.91 ± 0.34	1.10 ± 0.62	1.09 ± 0.61
		Polyunsaturated	0.86 ± 0.36	0.57 ± 0.25	0.83 ± 0.48
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	0.54 ± 0.01	0.77 ± 0.16	0.60 ± 0.15
		Polyunsaturated	2.27 ± 2.09	1.49 ± 1.22	1.09 ± 0.78
Dexamethasone	Jejunum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.06 ± 0.34	1.13 ± 0.22	1.13 ± 0.17
		Polyunsaturated	1.10 ± 0.15	0.83 ± 0.18	0.93 ± 0.23
	Ileum	Chow	1 ± 0	1 ± 0	1 ± 0
		Saturated	1.34 ± 0.89	0.95 ± 0.30	0.77 ± 0.18
		Polyunsaturated	1.23 ± 0.09	1.12 ± 0.23	1.10 ± 0.40

Mean ± SD

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 81. Effect of Steroids on the Expression of SGLT1, Na^+/K^+ ATPase Alpha 1 and Beta 1

Diet		Drug	SGLT1	Alpha 1	Beta 1
Chow	Jejunum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.66 ± 0.65	0.81 ± 0.15	1.02 ± 0.07
		Prednisone	0.73 ± 0.52	0.40 ± 0.51	1.08 ± 1.28
		Dexamethasone	1.08 ± 0.15	0.45 ± 0.62	0.89 ± 0.98
	Ileum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.91 ± 0.37	4.22 ± 2.55	1.39 ± 0.02*
		Prednisone	1.18 ± 0.18	2.00 ± 2.25	0.93 ± 0.19~
		Dexamethasone	0.79 ± 0.20	3.44 ± 1.15	1.29 ± 0.03*^
Saturated	Jejunum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.73 ± 0.63	0.95 ± 0.32	1.39 ± 0.94
		Prednisone	0.76 ± 0.37	0.72 ± 0.13	1.03 ± 0.51
		Dexamethasone	1.15 ± 0.86	0.87 ± 0.61	1.47 ± 0.17
	Ileum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.52 ± 0.38	0.53 ± 0.56	0.97 ± 0.47
		Prednisone	1.98 ± 1.46	0.67 ± 0.29	0.73 ± 0.26
		Dexamethasone	1.92 ± 0.64	0.81 ± 0.31	0.58 ± 0.28
Polyunsaturated	Jejunum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	1.27 ± 1.18	6.28 ± 8.28	1.34 ± 0.16
		Prednisone	1.22 ± 1.32	11.94 ± 16.88	0.91 ± 0.45
		Dexamethasone	0.52 ± 0.07	9.75 ± 10.95	0.97 ± 0.48
	Ileum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	2.42 ± 1.69	0.22 ± 0.31*	1.89 ± 0.79
		Prednisone	3.40 ± 1.82	0.05 ± 0.06*	8.24 ± 10.94
		Dexamethasone	2.66 ± 1.11	0.02 ± 0.03*	8.14 ± 11.50

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

Table 82. Effect of Steroids on the mRNA for SGLT1, Na^+/K^+ ATPase Alpha 1 and Beta1

Diet		Drug	SGLT1	Alpha 1	Beta 1
Chow	Jejunum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.80 ± 0.53	1.34 ± 0.72	1.24 ± 0.72
		Prednisone	1.31 ± 1.24	2.20 ± 2.06	2.34 ± 2.53
		Dexamethasone	0.83 ± 0.63	1.08 ± 0.48	1.18 ± 0.59
	Ileum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.87 ± 0.37	0.83 ± 0.17	0.86 ± 0.12
		Prednisone	1.23 ± 0.50	0.96 ± 0.12	0.97 ± 0.11
		Dexamethasone	0.79 ± 0.03	0.88 ± 0.11	0.85 ± 0.17
Saturated	Jejunum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	1.00 ± 0.09	0.94 ± 0.06	1.02 ± 0.19
		Prednisone	1.18 ± 0.30	1.37 ± 0.30~	1.30 ± 0.14
		Dexamethasone	1.09 ± 0.35	1.12 ± 0.26	1.17 ± 0.24
	Ileum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.89 ± 0.69	1.95 ± 2.65	0.76 ± 0.19
		Prednisone	0.60 ± 0.22	1.69 ± 2.24	0.64 ± 0.24*
		Dexamethasone	0.94 ± 0.09	2.15 ± 3.11	0.68 ± 0.14*
Polyunsaturated	Jejunum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	0.79 ± 0.33	0.87 ± 0.32	0.86 ± 0.41
		Prednisone	0.76 ± 0.31	0.65 ± 0.16	0.83 ± 0.38
		Dexamethasone	0.85 ± 0.43	0.68 ± 0.19	0.77 ± 0.30
	Ileum	Control	1 ± 0	1 ± 0	1 ± 0
		Budesonide	1.16 ± 0.28	1.67 ± 0.60	1.59 ± 0.49
		Prednisone	2.69 ± 2.20	1.76 ± 1.38	1.64 ± 0.86
		Dexamethasone	1.17 ± 0.22	1.27 ± 0.34	1.33 ± 0.41

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

Table 83. Effect of Resection on the expression of Expression of SGLT1, Na$^+$/K$^+$ ATPase Alpha 1 and Beta1

	Jejunum			Ileum		
Diet	SGLT1	alpha1	beta1	SGLT1	alpha1	beta1
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	1.08 ± 0.06	1.19 ± 0.35	1.72 ± 0.58	1.24 ± 0.07+	0.64 ± 0.18+	0.63 ± 0.48

Mean ± SD

+, $p<0.05$, transection vs resection

Table 84. Effect of Resection on the mRNA for SGLT1, Na^+/K^+ ATPase Alpha 1 and Beta1

	Jejunum			Ileum		
Diet	SGLT1	alpha1	beta1	SGLT1	alpha1	beta1
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	0.85 ± 0.38	0.59 ± 0.28+	0.63 ± 0.29+	0.99 ± 0.09	0.96 ± 0.24	0.91 ± 0.24

Mean ± SD

+, $p<0.05$, transection vs resection

Table 85. Effect of Diets on the Expression of GLUT5 and GLUT2

Drug	Diet	Jejunum		Ileum	
		GLUT5	GLUT2	GLUT5	GLUT2
Control	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.78 ± 0.10	1.37 ± 0.41	1.17 ± 0.33	1.65 ± 1.21
	Polyunsaturated	1.02 ± 0.35	0.93 ± 0.65	1.50 ± 0.88	0.39 ± 0.18
Budesonide	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	1.07 ± 0.19	1.39 ± 0.87	0.70 ± 0.05&	1.42 ± 0.86
	Polyunsaturated	0.96 ± 0.33	0.50 ± 0.60	0.88 ± 0.04&#	1.09 ± 1.25
Prednisone	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	1.38 ± 0.31	0.85 ± 0.27	1.25 ± 0.91	0.74 ± 0.62
	Polyunsaturated	1.41 ± 0.50	0.59 ± 0.35	2.04 ± 1.54	0.76 ± 0.30
Dexamethasone	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	1.10 ± 0.30	1.01 ± 0.74	1.34 ± 0.33	0.48 ± 0.39&
	Polyunsaturated	1.32 ± 0.46	0.69 ± 0.48	1.86 ± 0.62	0.24 ± 0.16&

Mean ± SD

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 86. Effect of Diets on the mRNA Expression for GLUT5 and GLUT2

Drug	Diet	Jejunum		Ileum	
		GLUT5	GLUT2	GLUT5	GLUT2
Control	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	1.07 ± 0.01	0.87 ± 0.42	0.63 ± 0.05&	0.92 ± 0.33
	Polyunsaturated	0.99 ± 0.30	1.40 ± 0.83	0.92 ± 0.07#	0.72 ± 0.23
Budesonide	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.87 ± 0.01	0.87 ± 0.23	0.89 ± 0.01	0.91 ± 0.12
	Polyunsaturated	0.98 ± 0.19	0.96 ± 0.06	0.94 ± 0.36	0.87 ± 0.40
Prednisone	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.61 ± 0.11&	1.11 ± 0.68	0.79 ± 0.04	0.76 ± 0.11
	Polyunsaturated	0.46 ± 0.09&	0.93 ± 0.62	2.16 ± 1.73	0.58 ± 0.31&
Dexamethasone	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	1.04 ± 0.02	1.15 ± 0.26	0.77 ± 0.31	0.76 ± 0.28
	Polyunsaturated	1.07 ± 0.02	1.03 ± 0.06	0.83 ± 0.14	0.76 ± 0.32

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 87. Effect of Steroids on the Expression of GLUT5 and GLUT2

Diet	Drug	Jejunum		Ileum	
		GLUT5	GLUT2	GLUT5	GLUT2
Chow	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	1.69 ± 1.22	0.92 ± 0.30	0.74± 0.65	0.96 ± 0.33
	Prednisone	1.50 ± 1.22	0.92 ± 0.52	0.85 ± 0.16	1.72 ± 0.74
	Dexamethasone	1.43 ± 1.03	0.92± 0.24	0.74 ± 0.18	2.02 ± 0.94
Saturated	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	2.40± 1.98	0.84 ± 0.13	0.64 ± 0.39	0.86 ± 0.17
	Prednisone	2.35 ± 1.37	0.57± 0.31	0.85 ± 0.45	0.80 ± 0.73
	Dexamethasone	1.85 ± 1.05	0.53 ± 0.28	0.85 ± 0.18	0.61 ± 0.50
Polyunsaturated	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	1.52 ± 0.95	0.44 ± 0.58	0.99 ± 0.05	2.47 ± 1.80
	Prednisone	1.92±1.33	0.60 ± 0.23	1.07 ± 0.12	3.51 ± 1.77
	Dexamethasone	1.82 ± 1.27	0.75 ± 0.32	0.97 ± 0.14	0.99 ± 0.41

Mean ± SD

None of these differences was statistically significant.

Table 88. Effect of Steroids on the mRNA Expression for GLUT5 and GLUT2

Diet	Drug	Jejunum		Ileum	
		GLUT5	GLUT2	GLUT5	GLUT2
Chow	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	1.18 ± 0.05	1.03 ± 0.59	0.89 ± 0.01	0.83 ± 0.17
	Prednisone	2.21 ± 0.27*~	1.96 ± 2.15	0.87 ± 0.01	0.96 ± 0.12
	Dexamethasone	1.09 ± 0.01^	0.93 ± 0.46	0.98 ± 0.25	0.88 ± 0.11
Saturated	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	0.96 ± 0.04	0.95 ± 0.02	1.26 ± 0.10	1.95 ± 2.65
	Prednisone	1.24 ± 0.05*~	1.27 ± 0.31	1.10 ± 0.04	1.69 ± 2.24
	Dexamethasone	1.06 ± 0.01^	1.26 ± 0.47	1.14 ± 0.08	2.15 ± 3.11
Polyunsaturated	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	1.18 ± 0.09	0.77 ± 0.33	0.92 ± 0.41	1.06 ± 0.42
	Prednisone	1.04 ± 0.23	0.76 ± 0.41	2.11 ± 1.81	0.84 ± 0.55
	Dexamethasone	1.23 ± 0.36	0.80 ± 0.42	0.86 ± 0.01	0.99 ± 0.24

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

Table 89. Effect of Resection on the Expression of GLUT5 and GLUT2

	Jejunum		Ileum	
Diet	GLUT5	GLUT2	GLUT5	GLUT2
Transected				
	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected				
	1.05 ± 0.26	1.77 ± 0.95	0.94 ± 0.08	1.40 ± 0.56

Mean ± SD

None of these differences was statistically significant.

Table 90. Effect of Resection on the mRNA for GLUT5 and GLUT2

	Jejunum		Ileum	
Diet	GLUT5	GLUT2	GLUT5	GLUT2
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	0.77 ± 0.05+	0.71 ± 0.30	0.82 ± 0.23	0.93 ± 0.14

Mean ± SD

+, $p<0.05$, transection vs resection

Table 91. Effect of Diets on the ERG Expresssion

Drug		Diet	c-jun	c-myc	c-fos
Control	Jejunum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	0.53 ± 0.09	1.04 ± 0.87	N.S.
		Polyunsaturated	0.57 ± 0.33	1.29 ± 0.34	N.S.
	Ileum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	0.67 ± 0.17	0.38 ± 0.29	N.S.
		Polyunsaturated	0.80 ± 025	0.90 ± 0.05	N.S.
Budesonide	Jejunum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	1.16 ± 0.02&	0.80 ± 0.23	N.S.
		Polyunsaturated	1.06 ± 0.04&#	0.88 ± 0.35	N.S.
	Ileum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	0.79 ± 0.18	0.91 ± 0.19	N.S.
		Polyunsaturated	1.35 ± 0.52	1.28 ± 0.67	N.S.
Prednisone	Jejunum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	1.22 ± 0.22	3.03 ± 1.23	N.S.
		Polyunsaturated	1.08 ± 0.09	0.59 ± 0.13	N.S.
	Ileum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	0.56 ± 0.26	0.52 ± 0.09	N.S.
		Polyunsaturated	1.52 ± 1.28	2.12 ± 2.13	N.S.
Dexamethasone	Jejunum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	0.98 ± 0.10	0.86 ± 0.01	N.S.
		Polyunsaturated	0.81 ± 0.17	0.99 ± 0.40	N.S.
	Ileum	Chow	1 ± 0	1 ± 0	N.S.
		Saturated	0.97 ± 0.75	0.97 ± 0.67	N.S.
		Polyunsaturated	0.78 ± 0.20	0.75 ± 0.01	N.S.

Mean ± SD

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

N.S.= no signal

Table 92. Effect of Steroids on the ERG Expresssion

Diet		Drug	c-jun	c-myc	c-fos
Chow	Jejunum	Control	1 ± 0	1 ± 0	N.S.
		Budesonide	0.43 ± 0.02*	1.08 ± 0.70	N.S.
		Prednisone	0.46 ± 0.02*	1.84 ± 1.83	N.S.
		Dexamethasone	0.54 ± 0.11*	1.03 ± 0.79	N.S.
	Ileum	Control	1 ± 0	1 ± 0	N.S.
		Budesonide	0.99 ± 0.54	0.83 ± 0.23	N.S.
		Prednisone	1.43 ± 1.00	1.22 ± 0.49	N.S.
		Dexamethasone	1.02 ± 0.18	1.06 ± 0.14	N.S.
Saturated	Jejunum	Control	1 ± 0	1 ± 0	N.S.
		Budesonide	0.95 ± 0.16	0.90 ± 0.02	N.S.
		Prednisone	1.05 ± 0.07	5.08 ± 3.26	N.S.
		Dexamethasone	1.00 ± 0.09	0.89 ± 0.10	N.S.
	Ileum	Control	1 ± 0	1 ± 0	N.S.
		Budesonide	1.08 ± 0.05	2.75 ± 2.21	N.S.
		Prednisone	0.99 ± 0.23	2.49 ± 2.27	N.S.
		Dexamethasone	1.44 ± 0.94	4.39 ± 4.82	N.S.
Polyunsaturated	Jejunum	Control	1 ± 0	1 ± 0	N.S.
		Budesonide	0.97 ± 0.45	0.76 ± 0.57	N.S.
		Prednisone	1.09 ± 0.56	0.69 ± 0.47	N.S.
		Dexamethasone	0.91 ± 0.40	0.82 ± 0.71	N.S.
	Ileum	Control	1 ± 0	1 ± 0	N.S.
		Budesonide	1.57 ± 0.61	1.08 ± 0.23	N.S.
		Prednisone	2.33 ± 1.59	2.25 ± 1.61	N.S.
		Dexamethasone	1.00 ± 0.10	0.87 ± 0.06	N.S.

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

N.S.= no signal

Table 93. Effect of Resection on the ERG Expression

	Jejunum			Ileum		
Diet	c-jun	c-myc	c-fos	c-jun	c-myc	c-fos
Transected						
	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0	N.S
Resected						
	0.63 ± 0.45	0.77 ± 0.35	N.S	0.83 ± 1.09	0.94 ± 0.19	N.S

Mean ± SD

None of these differences was statistically significant.

Table 94. Effect of Diets on the Proglucagon and ODC mRNA Expression

Drug	Diet	Jejunum		Ileum	
		Prog	ODC	Prog	ODC
Control	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.41 ± 0.01&	0.65 ± 0.34	0.94 ± 0.36	0.76 ± 0.34
	Polyunsaturated	1.02 ± 0.12#	1.94 ± 2.25	0.96 ± 0.04	0.75 ± 0.23
Budesonide	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.71 ± 0.11	0.65 ± 0.10&	0.93 ± 0.02	0.87 ± 0.06
	Polyunsaturated	0.96 ± 0.24	0.87 ± 0.15#	0.94 ± 0.25	1.28 ± 0.51
Prednisone	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.73 ± 0.10	0.97 ± 0.37	0.79 ± 0.03	0.80 ± 0.21
	Polyunsaturated	0.98 ± 0.15	0.67 ± 0.29	3.31 ± 0.12&#	2.10 ± 1.40
Dexamethasone	Chow	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.89 ± 0.06	1.06 ± 0.15	1.00 ± 0.10	0.80 ± 0.29
	Polyunsaturated	1.01 ± 0.13	0.97 ± 0.19	1.10 ± 0.10	0.98 ± 0.27

Mean ± SD

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 95. Effect of Steroids on the Proglucagon and ODC mRNA Expression

Diet	Drug	Jejunum		Ileum	
		Prog	ODC	Prog	ODC
Chow	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	0.49 ± 0.08*	1.02 ± 0.49	1.01 ± 0.10	0.81 ± 0.20
	Prednisone	0.46 ± 0.08*	1.12 ± 0.72	1.01 ± 0.18	0.84 ± 0.11
	Dexamethasone	0.44 ± 0.01*	0.78 ± 0.24	0.88 ± 0.22	0.82 ± 0.05
Saturated	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	0.96 ± 0.04	1.03 ± 0.20	1.26 ± 0.10	0.98 ± 0.15
	Prednisone	1.24 ± 0.05*~	1.58 ± 0.38	1.10 ± 0.04	0.94 ± 0.26
	Dexamethasone	1.06 ± 0.01^	1.39 ± 0.38	1.14 ± 0.08	0.91 ± 0.21
Polyunsaturated	Control	1 ± 0	1 ± 0	1 ± 0	1 ± 0
	Budesonide	1.18 ± 0.09	0.77 ± 0.33	0.92 ± 0.41	1.06 ± 0.42
	Prednisone	1.04 ± 0.23	0.76 ± 0.41	2.11 ± 1.81	0.84 ± 0.55
	Dexamethasone	1.23 ± 0.36	0.80 ± 0.42	0.86 ± 0.01	0.99 ± 0.24

Mean ± SD

*, $p<0.05$, budesonide, prednisone or dexamethasone vs control

~, $p<0.05$, prednisone or dexamethasone vs budesonide

^, $p<0.05$, dexamethasone vs prednisone

Table 96. Effect of Resection on the the Proglucagon and ODC mRNA Expression

	Jejunum		Ileum	
Diet	Prog	ODC	Prog	ODC
Transected	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Resected	1.10 ± 0.09	0.52 ± 0.12+	0.98 ± 0.11	0.85 ± 0.16

Mean ± SD

+, $p<0.05$, transection vs resection

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N) DIETARY LIPIDS ALTER THE EFFECT OF STEROIDS ON THE UPTAKE OF LIPIDS FOLLOWING INTESTINAL RESECTION IN RATS

1) Introduction

The topic of intestinal adaptation has been reviewed [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, there is hyperplasia of the remaining intestine which may be accompanied by enhanced uptake of nutrients [Dowling and Booth, 1967; Garvey et al., 1976; Hanson et al., 1977; King et al., 1981; Kinter and Wilson, 1975]. The signals which mediate this adaptive process are incompletely understood, but may include proglucagon-derived peptides, ornithine decarboxylase (ODC), and early response genes (ERG) [Bloom and Polak, 1982; Bristol and Williamson, 1988; Rountree et al., 1992; Sagor et al., 1983; Tappenden et al., 1998]. ERG such as c-myc, c-jun and c-fos have been demonstrated to be involved in processes of proliferation and differentiation, as well as ODC, a key enzyme in the synthesis of polyamines and a requirement in any proliferative event [Sagor et al., 1983; Tappenden et al., 1996]. Proglucagon has been shown to be involved in or at least to contribute to the intestinal adaptive process [Mojsov et al., 1986; Orskov et al., 1987]. Short chain fatty acids increase the ileal c-myc and proglucagon expression in rats undergoing intestinal resection [Tappenden and McBurney, 1998]. The adaptation in lipid absorption following intestinal resection must involve other signals. Epimorphin/syntaxin 2 mRNA, that codes for a membrane-associated protein involved in morphogenesis of the lungs and skin, as well as PC4/TIS7, a gene involved in nerve growth factor-mediated cytodifferentiation, may be among other signals that might be also involved in the adaptive response after intestinal resection [Goyal et al., 1998; Rubin et al., 1998]. New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001].

Isocaloric modification of the type of lipids in the diet is associated with changes in nutrient uptake in models of intestinal adaptation such as diabetes, abdominal irradiation, chronic intake of ethanol, or aging [Thomson et al., 1989; Thomson et al. 1991; Thomson et al., 1987; Thomson et al., 1988]. For example, sugar and lipid uptake is increased in adult rats fed an isocaloric saturated (SFA) as compared with a polyunsaturated fatty acid (PUFA) diet [Thomson et al., 1986].

Systemically active glucocorticosteroids given by mouth enhance the intestinal absorption of sugars [Batt and Scott, 1982; Scott et al., 1980], and accelerate the early development of the intestine [Lebenthal e al., 1972]. The locally acting steroid budesonide is useful to treat patients with Crohn's disease [Brattsand, 1990; Campieri et al., 1995; Greenberg, 1994; Lofberg et al., 1993; Rutgeerts et al., 1994; Thiesen and Thomson, 1996]. In young rats with an intact intestinal tract budesonide enhances the intestinal uptake of fructose and some lipids [Thiesen et al., 1996]. While the injection of dexamethasone alters the DNA content of the bowel following intestinal resection [Park et al., 1994], it is unknown what is its effect on nutrient absorption.

Intestinal lipid uptake occurs by a process of passive permeation [Thomson et al., 1982], but a component of the uptake of long-chain fatty acids may also be mediated by the sodium/hydrogen exchanger and/or by lipid binding proteins in the brush border membrane or in the cytosol of the enterocyte, such as the liver fatty acid binding protein (L-FABP) or the ileal lipid binding protein (ILBP)[Thomson et al., 1996; Thomson and

Wild, 1997; Schoeller et al., 1995a; Schoeller et al., 1995b]. It is unknown if the lipid binding proteins in the intestine are influenced by steroids, dietary lipids or by resection.

Accordingly, these studies were undertaken to test the hypotheses that 1) the desired intestinal adaptive response following intestinal resection may be enhanced further by the administration of the locally active steroid budesonide or the systemically active prednisone or dexamethasone, and by feeding a saturated as compared with a polyunsaturated fatty acid diet; and 2) any alteration in lipid uptake is associated with changes in the expression of selected signals as well as the expression of the mRNAs for L-FABP and ILBP.

2) Methods and Materials

2.1) Northern Immunoblotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E.coli*) were transformed with plasmids containing the desired DNA sequences to be used as a probe for the Northern blotting. Early response gene (ERG) probes were obtained from Oncogene Research Products; the cDNA probe encoding proglucagon was obtained from Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; the ODC probe was obtained from Dr. Blackshear, University of Chicago; and the L-FABP and ILBP probes were provided by Dr. Agellon, University of Alberta. A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labelled DNA (Roche Diagnostics, Quebec, CA) that was dotted on the same membrane.

RNA was extracted from the jejunum and ileum of at least three animals in each of the four groups. These segments were homogenized in a denaturing solution containing guanidinium thiocyanate, using the biorad fast prep shaking centrifuge (BIO101, Vista, California, USA). Following addition with 2 M sodium acetate, a phenol chloroform extraction was performed. The upper aqueous phase was transferred to a tube, and the RNA was precipitated with isopropanol and washed with 70% ethanol.

Total RNA was separated, based on molecular weight as it was electrophoresed through a denaturing agarose gel (1.16% agarose). RNA was then transferred form the gel to a nylon membrane by capillary action overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA.

As a pre-hybridization, membranes were incubated for30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at 42°C overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid) in order to reduce non-specific binding sites. They were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was done using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA), and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. Thus, the density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA). To determine the exact RNA quantity that had been loaded, the 28 S ribosomal band density on the membrane was evaluated after the transfer. At the end of the experiment, the RNA band density of the film was also evaluated. The ratio between the RNA band density value on the film and on the membrane was calculated.

3) Results

3.1) Animal Characteristics

In transected rats, food intake was higher when the animals were fed with chow than with the saturated fatty acid [SFA] or polyunsaturated fatty acid diets [PUFA] (Table 69). Body weight gain and weight gain per food intake were lower with SFA than with PUFA or chow. In resected animals fed PUFA, both weight gain and weight gain per food intake were lower than in transected animals fed PUFA, but were similar in resected rats fed PUFA as compared with SFA or chow.

Dexamethasone reduced body weight gain in resected animals fed chow, SFA or PUFA (Table 70). Only in PUFA was this associated with reduced food intake. In contrast, budesonide and prednisone had no effect on food intake or body weight gain in rats fed chow, SFA or PUFA. Resected rats given budesonide or prednisone gained more weight when fed PUFA, as compared with those fed SFA.

In transected rats, the total weight of the intestine and the percentage of the intestinal wall comprised of scrapable mucosa was similar in the jejunum and in the ileum of rats fed chow, SFA or PUFA (Table 71). The weight of the jejunum was greater in resected than in transected rats fed PUFA, but the percentage of the intestinal wall comprised of mucosa was less. There were no difference in the intestinal characteristics of resected versus transected animals fed chow or SFA.

Giving budesonide, prednisone or dexamethasone to rats fed chow, SFA or PUFA had no effect on the weight per centimeter of intestine remaining after resection, or on the percentage of the intestinal wall comprised of mucosa (Table 72). The weight of the jejunal wall was approximately one-third lower in resected rats given budesonide (16.6 ± 1.3) or prednisone (16.3 ± 2.4) and fed PUFA, as compared with those fed SFA or chow (23.1 ± 2.3 and 24.4 ± 1.8, and 24.2 ± 1.7 and 24.4 ± 1.8, respectively). The percentage of the intestinal wall comprised of mucosa was increased in animals given dexamethasone and fed PUFA (65.6 ± 3.1 vs 56.3 ± 4.1).

3.2) Lipid Uptake

Intestinal resection had no effect on lipid uptake into the jejunum or ileum of rats fed chow (Fig. 12). There was lower jejunal uptake of 16:0 in resected than in transected rats fed SFA. In resected as compared with transected rats fed PUFA, there was higher jejunal uptake of 16:0 and 18:2, and lower ileal uptake of cholesterol. In transected rats fed PUFA, jejunal uptake of 16:0 and 18:2 were lower than in rats fed chow, as well as the jejunal uptake of 18:2 in transected rats fed SFA. In the transected rats fed SFA, ileal uptake of cholesterol was lower than irats fed chow or PUFA.

Steroids had no effect on the jejunal or ileal uptake of 16:0, 18:2, or cholesterol in resected rats fed chow, SFA or PUFA (Table 97). Dietary lipids had no effect on the uptake of long-chain fatty acids or cholesterol in rats given prednisone. The jejunal uptake of 18:2 was lower in rats given budesonide or dexamethasone, and fed SFA or PUFA as compared with those fed chow. The jejunal uptake of 16:0 and 18:2 was reduced in resected animals given control vehicle and fed SFA or PUFA as compared to those fed chow; as well, the ileal uptake of 16:0 in those fed SFA was lower than in those fed chow. The ileal uptake of 18:2 was increased in animals given control vehicle and fed PUFA as compared to those fed chow and SFA, whereas the jejunal uptake of 18:2 was reduced in animals given budesonide and fed PUFA.

3.3) Early Response Gene Expression

Dietary lipids did not alter the expression of the early response gene c-myc, but SFA and PUFA increased the expression of c-jun in the jejunum of resected rats given budesonide as compared to those fed chow (Table 91). No differences in the expression of c-jun were observed in the ileum. Budesonide, prednisone and dexamethasone reduced the jejunal expression of c-jun in resected animals fed chow, but had no effect in animals fed SFA or PUFA (Table 92). There were no differences in the expression of c-myc and c-jun in resected as compared to transected animals (Table 93).

3.4) Proglucagon and ODC Expression

In resected rats given control vehicle, there was a reduction in jejunal proglucagon mRNA expression in those fed SFA as compared to those fed chow or PUFA. The animals given budesonide and fed SFA had a reduction in jejunal ODC mRNA expression as compared to those fed chow or PUFA (Table 94). In the ileum, ODC expression was unaffected by steroids or dietary lipids, and proglucagon expression was increased in those animals fed PUFA and given prednisone as compared to those fed SFA or chow (Table 94).

Steroids reduced the jejunal but not the ileal expression of the mRNA for proglucagon in resected animals fed chow (Table 95). Prednisone given to rats fed SFA, increased the jejunal expression of the mRNA for proglucagon. ODC mRNA expression was not affected by steroids or by modifying dietary lipids.

Proglucagon expression in the jejunum and ileum was not changed by resection (Table 96). ODC expression in the jejunum of resected animals was reduced as compared with transection. No changes in the ileal expression of ODC was observed between resected and transected animals.

3.5) L-FABP and ILBP Expression

As expected, the ileal lipid binding protein (ILBP) mRNA was present only in the ileum. Its abundance was unaffected by steroids, dietary lipids or resection (Table 98). The liver fatty acid binding protein (L-FABP) mRNA was also unaffected by steroids (Table 99) and by dietary lipids, but resection reduced the jejunal expression of L-FABP mRNA (Table 100).

4) Discussion

Glucocorticosteroids are used to treat patients with a variety of intestinal conditions, including Crohn's disease and ulcerative colitis. Recent clinical attention has focussed on the use of potent locally acting steroids such as budesonide [Campieri et al., 1995; Greenberg et al., 1994; Lofberg et al., 1993]. Prednisone and budesonide modify the morphology and absorptive function of the intestine in young rats with an intact intestinal tract [Thiesen et al., 1996]. This study extends these observations to compare the influence of the locally active budesonide versus two systemically active steroids (prednisone given by mouth, and dexamethasone given by subcutaneous injection) in animals in which a portion of the jejunum and ileum remained after removal of the middle half of the small intestine.

The intestinal absorption of nutrients is subject to adaptation of mediated- and non-mediated processes, with alterations of transport influenced by the animal's age, diet, as well as by pathological processes such as diabetes mellitus, chronic ethanol intake and abdominal irradiation [Thomson et al., 1996; Thomson and Wild, 1997]. Following intestinal resection, morphological and functional changes occur depending upon the extent of the intestine removed, the site studied, and the lipid content of the diet [Thomson and O'Brien, 1981; Thomson et al., 1991; Thomson et al., 1989; Thomson et al., 1987; Thomson et al., 1988]. The signals for this process are unknown, but may include proglucagon-derived peptides, ERGs and ODC [Bloom and Polak, 1982; Bristol and Williamson, 1988; Rountree et al., 1992; Sagor et al., 1983; Tappenden et al., 1998; Reimer and McBurney, 1996]. In this study, we have shown involvement of the early response gene c-jun, as well as proglucagon and ODC in this adaptive response. For example, animals fed SFA and given budesonide had a reduction in jejunal ODC mRNA expression as compared to those fed chow or PUFA, and animals fed SFA and given prednisone had an enhancement in jejunal expression of proglucagon mRNA (Table 95). These results did not correlate with the uptake findings (Table 97). It was initially speculated that up-regulation of the signals for ERG, ODC and proglucagon would result in phenotype alterations that would lead to changes in transport activity. In fact, these signals either do not change, are down-regulated in some instances or up-regulated in others, and do not correlate with transport activity. This suggests the involvement of other signals in this adaptive process. New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis [Erwin et al., 2000; Stern et al., 2001].

The systemic administration of dexamethasone blunts the morphological adaptation of the intestine of rats when studied one week after an 80% enterectomy [Park et al., 1994]. We used young rats studied two weeks after removal of the middle half of the small intestine (50% enterectomy). The extent of resection is known to result in morphological and functional adaptation [Keelan et al., 1996], and a 50% enterectomy rather than a massive resection was chosen because sufficient proximal and distal intestine remained to assess the morphology and function at both sites. Furthermore, a 50% resection does not usually result in body weight loss [Keelan et al., 1996], and is closer to the clinical situation seen, for example, in some patients with Crohn's disease. In this study, food intake and body weight gain were unaffected by this 50% intestinal resection in rats fed chow or SFA (Table 69). Food intake was similar in transected and resected rats fed PUFA, but weight gain was lower in resected than in transected animals. The explanation for this lower weight gain is unclear, since the jejunal uptake of 16:0 and 18:2 was in fact higher in resected animals fed PUFA as compared to transected animals (Fig. 11).

In resected animals, neither budesonide nor prednisone altered the weight of the intestine, or the percentage of the intestinal wall comprised of mucosa. It is possible that had we subjected the animals to a massive bowel resection (eg, 80% of the small intestine), then the weight of the remaining intestine per unit length or surface area might have changed. Nonetheless, based on this data two weeks following a 50% enterectomy, it is clear that these steroids given in these doses and by these routes have no adverse effects on the weight of the intestine. A similar lack of effect of budesonide and prednisone on intestinal weight has been reported in non-operated intestine [Thiesen et al., 1996]. Furthermore, this data underline the point that the effects of steroids on the absorptive functions of the intestine, to be discussed below, are not due to any steroid-induced change in the weight of the intestine.

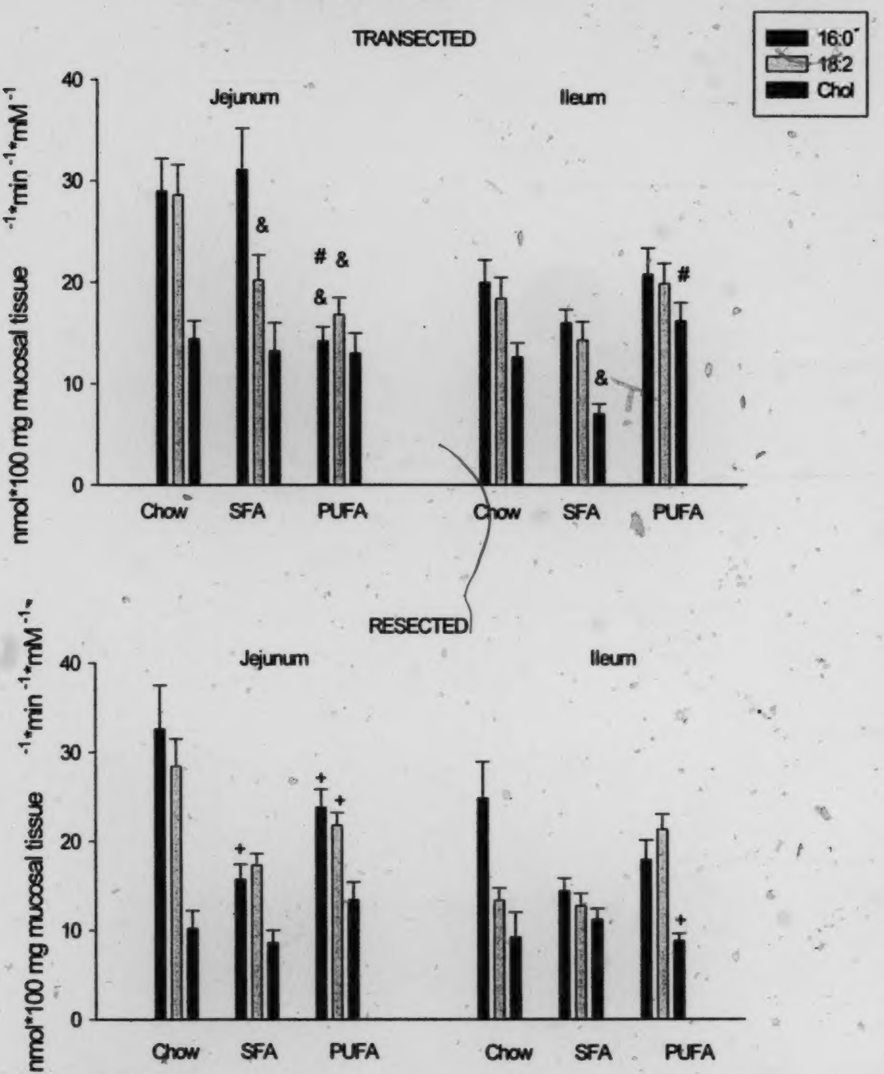
While the absorption of nutrients may be increased after intestinal resection, the magnitude of this effect depends on the extent of resection, as well as on the site and time after surgery when experiments are performed [Hanson et al., 1977; Williamson et al., 1978; Williamson, 1982a; Williamson, 1982b]. We could have subjected the animals to a massive small bowel resection, and potentially been able to show enhanced uptake of sugars and lipids. However, the interpretation of the results then would have been influenced and been made difficult by the expected concomitant changes in food intake, body weight gain and intestinal weight. Furthermore, we wished these studies to have some potential clinical relevance. For example, in patients with Crohn's disease, prednisone or budesonide may be used therapeutically in the doses used here, and while these individuals previously may have undergone an intestinal resection, massive resections would be unusual.

Isocaloric changes in dietary lipids modify the intestinal absorption of nutrients in adult rats, with greater uptake of sugars, amino acids and fatty acids in animals fed a semisynthetic saturated (SFA) versus polyunsaturated (PUFA) diets [Thomson et al., 1982]. This observation has been applied to modify the adaptation of the intestine in a manner which achieves a therapeutic objective. For example, feeding SFA enhances uptake and prevents or reduces the malabsorption which otherwise would occur following the chronic intake of ethanol [Thomson et al, 1991], or following abdominal irradiation [Thomson et al., 1989]. Furthermore, the undesired enhanced uptake of sugars and lipids which occurs in experimental diabetes mellitus may be prevented by feeding PUFA [Thomson et al., 1987]. This has the additional clinical advantage of improving glucose control and reducing the hyperlipidemia and elevated concentrations of hemoglobin A1c [Thomson et al., 1987; Thomson et al., 1988; Keelan et al., 1989].

None of these three steroids affected the jejunal or ileal uptake of palmitic (16:0) or linoleic acids (18:2) or cholesterol in resected rats fed chow, SFA or PUFA (Table 97). The adaptation of intestinal uptake of lipids is associated with changes in the intestinal brush border membrane and enterocyte microsomal membrane phospholipids or phospholipid fatty acids [Garg et al., 1988; Garg et al., 1990; Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1986; Keelan et al., 1987; Keelan et al., 1993], as well as some phospholipid and fatty acid metabolizing enzymes [Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1986]. The only exception to this general rule has been the lack of effect of changes in dietary lipids on the phospholipids in the brush border membrane, which do demonstrate changes in lipid uptake under the same experimental conditions [Keelan et al., 1987]. In this study we did not measure brush border membrane lipids, so we are not able to comment upon whether the lack of effect of steroids on fatty acid uptake was due to their failure to affect the brush border membrane lipids or the protein-mediated component of uptake [Schoeller et al., 1995a; Schoeller et al., 1995b; Ibrahimi et al., 1996; Poirier et al., 1996]. Furthermore, steroids and dietary lipids did not modify the protein-mediated component of lipid uptake, as measured by the expression of the mRNAs for ILBP and L-FABP. The jejunal L-FABP mRNA was reduced in resected animals, but this did not contribute to any change in lipid uptake.

Recent support for a dissociation between intestinal lipid uptake and the abundance of lipid binding proteins comes from the observation that clones of mice with disrupted genes for the intestinal fatty acid binding protein (I-FABP) are viable, have elevated plasma triacylglycerol, and weigh more than do the wild variant [Vassielva et al., 2000]. It is appreciated that the study illustrates the effects of another lipid binding protein, I-FABP, which was not analyzed in this study. It is also important to remember that we looked at the lipid binding protein mRNA expression, and it is possible that alterations in the protein abundance could have been observed. In order to discard the involvement of lipid binding proteins in the response observed in resected animals, further studies assessing protein abundance and expression of other lipid binding proteins must be performed.

Figure 12. Effect of Dietary Lipids and Intestinal Resection on Uptake of Fatty Acids and Cholesterol



Mean ± SEM

The rate of uptake of lipids has the units nmol·100 mg mucosal tissue^{-1}·min^{-1}·mM^{1}.

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

+, $p<0.05$, transection vs resection

Table 97. Effect of Steroids on Uptake of Fatty Acids and Cholesterol In Resected Rats

Diet	Site and Lipid	Control Vehicle	Budesonide	Prednisone	Dexamethasone
Chow	Jejunum				
	16:0	32.6± 4.9	21.8 ± 2.8	30.3 ± 3.4	19.3 ± 1.9
	18:2	28.4 ± 3.1	27.3 ± 2.6	17.1 ± 2.8	29.2 ± 3.2
	Chol	10.2 ± 2.0	8.0 ± 1.4	9.2 ± 2.6	10.6 ± 1.4
	Ileum				
	16:0	24.8 ± 4.1	17.4 ± 2.1	20.8 ± 3.7	21.9 ± 2.4
	18:2	13.3 ± 1.4	20.2 ± 3.6	16.4 ± 3.1	17.5 ± 2.9
	Chol	9.2 ± 2.8	12.4 ± 2.0	11.4 ± 2.01	10.8 ± 1.2
Saturated	Jejunum				
	16:0	15.7 ± 1.7 &	22.0 ± 2.6	21.1 ± 2.0	25.5 ± 5.6
	18:2	17.3 ± 1.3 &	16.6 ± 1.4 &	18.1 ± 2.0	15.0 ± 1.9 &
	Chol	8.6 ± 1.4	10.0 ± 1.6	6.2 ± 0.8	13.0 ± 3.0
	Ileum				
	16:0	14.4 ± 1.4 &	13.4 ± 1.7	16.7 ± 2.7	20.2 ± 2.5
	18:2	12.7 ± 1.4	13.9 ± 1.5	13.3 ± 1.2	20.5 ± 3.6
	Chol	11.2 ± 1.2	9.4 ± 1.4	6.6 ± 0.6	10.0 ± 1.81
Polyunsaturated	Jejunum				
	16:0	23.8 ± 2.0 &	16.2 ± 1.7	21.7 ± 2.8	18.5 ± 2.2
	18:2	21.8 ± 1.4 &	15.1 ± 1.5 &	18.0 ± 3.4	19.0 ± 3.3 &
	Chol	13.4 ± 2.0	8.4 ± 2.01	11.8 ± 1.6	8.2 ± 1.4
	Ileum				
	16:0	17.9 ± 2.2	18.0 ± 1.9	16.4 ± 2.0	22.0 ± 3.6
	18:2	21.3 ± 1.7 &#	20.4 ± 2.6	19.60 ±2.9	17.0 ± 2.4
	Chol	8.8 ± 0.8	15.2 ± 3.0	8.8 ± 1.6	12.8 ± 1.8

Mean ± SEM

The rate of uptake of lipids has the units nmol·100 mg mucosal tissue^{-1}·min^{-1}·mM^{-1}.

#, $p<0.05$, polyunsaturated vs saturated fatty acid diet

&, $p<0.05$, saturated or polyunsaturated fatty acid diet vs chow diet

Table 98. Effect of Dietary Lipids on the L-FABP and ILBP mRNA Expression in Resected Rats

Drug	Diet	Jejunum	Ileum	
		L-FABP	L-FABP	ILBP
Control Vehicle	Chow	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.59 ± 0.48	1.07 ± 0.67	0.93 ± 0.25
	Polyunsaturated	2.05 ± 2.26	0.83 ± 0.36	0.90 ± 0.31
Budesonide	Chow	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.82 ± 0.05	0.87 ± 0.12	1.06 ± 0.16
	Polyunsaturated	0.97 ± 0.12	1.04 ± 0.32	1.32 ± 0.62
Prednisone	Chow	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.92 ± 0.28	0.59 ± 0.07	0.84 ± 0.12
	Polyunsaturated	0.76 ± 0.27	1.73 ± 1.34	2.17 ± 1.84
Dexamethasone	Chow	1 ± 0	1 ± 0	1 ± 0
	Saturated	0.84 ± 0.10	1.00 ± 0.55	0.78 ± 0.14
	Polyunsaturated	0.92 ± 0.24	1.04 ± 0.45	0.93 ± 0.33

Mean ± SD

None of these differences was statistically significant

Note that ILBP is not present in the jejunum

Table 99. Effect of Steroids on the L-FABP and ILBP mRNA Expression in Resected Rats

Diet	Drug	Jejunum	Ileum	
		L-FABP	L-FABP	ILBP
Chow	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
	Budesonide	1.06 ± 0.37	0.91 ± 0.17	0.88 ± 0.21
	Prednisone	1.39 ± 0.90	1.20 ± 0.29	1.05 ± 0.16
	Dexamethasone	1.06 ± 0.19	0.98 ± 0.22	1.08 ± 0.24
Saturated	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
	Budesonide	3.82 ± 5.00	0.74 ± 0.30	1.01 ± 0.12
	Prednisone	4.68 ± 5.95	0.65 ± 0.22	0.96 ± 0.17
	Dexamethasone	3.84 ± 4.88	0.79 ± 0.12	0.91 ± 0.15
Polyunsaturated	Control Vehicle	1 ± 0	1 ± 0	1 ± 0
	Budesonide	0.98 ± 0.68	0.96 ± 0.21	1.32 ± 0.67
	Prednisone	0.84 ± 0.56	2.05 ± 1.49	2.37 ± 1.73
	Dexamethasone	0.91 ± 0.58	1.03 ± 0.32	1.13 ± 0.31

Mean ± SD

None of these differences was statistically significant

Note that ILBP is not present in the jejunum

Table 100. Effect of Intestinal Resection on the L-FABP and ILBP mRNA Expression

	Jejunum		Ileum	
Diet	L-FABP	ILBP	L-FABP	ILBP
Transected				
	1 ± 0	N.S.	1 ± 0	1 ± 0
Resected				
	0.60 ± 0.14+	N.S.	0.74 ± 0.09	0.86 ± 0.15

Mean ± SD

+, $p<0.05$, transection vs resection

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O) DISCUSSION

1) Effects of Steroids

Animals fed BUD had a reduced rate of weight gain which was not explained by a change in food intake (Table 5), or in the rate of uptake of glucose (Table 7), fructose (Table 9) or lipids (Table 14). In fact, animals given BUD had increased intestinal uptake of fructose, and increased jejunal uptake of 18:1 and ileal uptake of 18:2 (Tables 9 and 14). The mechanism responsible for this lower weight gain in rats given BUD was not established in this study. However, in rats the hepatic metabolism is not as efficient as in humans (90% is metabolized to inactive metabolites and only 10% of intact budesonide is released at the systemic blood), allowing up to 25% of BUD to reach the systemic circulation after oral administration [Brattsand et al., 1990]. Furthermore, BUD has less of an effect on plasma cortisol concentration than does PRED. The most systemic activity of budesonide would then provide the expected negative effect on the weight gain. It is interesting to note that the dose of PRED used in this study did not alter body weight gain, despite its systemic nature. However, this effect of BUD on weight gain may have been spurious because 1) at higher doses (0.75 and 1.0 mg/kg), weight gain was similar to controls, and 2) BUD (0.25 mg/kg) has no effect on body weight gain in animals fed a semisynthetic diet enriched with saturated or polyunsaturated fatty acids (Table 18).

PRED acts both locally as well as systemically on the intestine, in contrast to the largely local site of action of BUD [Brattsand, 1990]. In adult animals, PRED increases glucose absorption [Batt and Peters, 1976a; Batt and Peters, 1976b; Batt and Scott, 1982]. The lack of effect of PRED on glucose uptake in this study may be due to the younger age of the animals (in this study the animals were post-weanings, and in the other studies the animals were adults). The lack of effect of PRED or BUD on the jejunal or ileal uptake of L-glucose or D-mannitol (Table 8) suggests that the paracellular contribution to sugar uptake also is unaffected by these steroids. The lack of effect of either PRED or BUD on the value of the Vmax of glucose uptake in these four week post-weaning rats (Table 7) suggests that there was no change in the expression of the activity of the sodium-dependent glucose transporter in the brush border membrane, SGLT1. In fact there was no change in either the abundance of the SGLT1 protein or mRNA expression (Tables 10 and 11). The linear relationship between fructose concentration and uptake precluded the calculation of values for Vmax or Km. We presume that this dose-response relationship would have become curvilinear had much higher concentration been used, but we selected to study concentrations which are commonly seen in the intestine human after a mixed meal [Wright EM et al., 1994]. Fructose uptake is mediated by GLUT5 (the sodium-independent fructose transporter in the brush border membrane). Fructose uptake was increased by steroids (Table 9), but there were no changes in the abundance of GLUT5 protein or mRNA expression. The possible explanations for this discrepancy are: 1) GLUT5 is activated by phosphorylation and/or glycosylation and our analyses do not differentiate between activated and non-activated GLUT5 (small differences in molecular weight). In this case the animals given steroids have more activated GLUT5 than the ones given saline or control vehicle. Against this possibility lies the factor that phosphorylation or glycosylation sites have not been described in the GLUT5 sequence [Burant et al., 1992] ; 2) GLUT5 is not evenly distributed along the crypt-villus unit so that more immature GLUT5 (at the crypt) is present in animals given saline and more mature GLUT5 (top 2/3 of the villus) is present in animals given steroids. This phenomenon is called "recruitment" and it was tested in a different set of animals and it will be discussed further; 3) if GLUT2 is truly present in the BBM as suggested by Kellet and collegues, it possibly could transport fructose into the enterocyte. In this case alterations in the abundance and expression of GLUT2 could explain the findings observed in animals given steroids. The abundance and expression of GLUT2 was assessed in the BLM and BBM. In the characteristic and well described typical location (BLM) we were able to get a strong signal for GLUT2 and that did not change with our treatment. Our attempts to replicate Kellet studies were not successful and at this point we could not confirm his findings.

Intestinal lipid uptake occurs mostly by a process of passive permeation [Thomson et al., 1996], but a component of the uptake of long-chain fatty acids also is mediated by the sodium/hydrogen exchanger [Schoeller et al., 1995a] and/or by fatty acid binding proteins in the brush border membrane [Poirier et al., 1996; Schoeller et al., 1995b]. The increased jejunal and ileal uptake of lipids in animals given BUD or PRED (Table 14) does not distinguish between which of these passive or mediated steps may have been affected by steroids. The enhanced uptake of lipids as a result of giving BUD or PRED was not explained by alterations in the animal's food intake or mucosal mass. Adaptations in lipid uptake may be due to alterations in the lipid content of the brush border membrane (BBM) [Keelan et a., 1996], but these measurements were not performed in this study. However, any steroid-associated change in the lipophilic properties of the BBM would be expected to modify the uptake of all lipids, and this did not occur. Thus, while it is unknown whether PRED or BUD affect the lipid composition of the BBM, we speculated that steroids also modify the protein-mediated component of lipid uptake. In order to test this hypothesis, we examined the expression of selected lipid binding proteins such as the liver-fatty acid binding protein (L-FABP) and the ileal lipid binding protein (ILBP). However, we were not able to show any increase of these proteins under circunstances where lipid uptake was increased. However, before abandoning the concept of the potential importance of lipid binding proteins in mediating the adaptation of lipid uptake, the role of other recently described lipid binding proteins must be examined.

The main reason to look at the mRNA for the transporters responsible for the absorption of sugars and lipids were to get clues regarding regulation of these proteins. If the activity of these transporters were up-regulated such as it happened for D-fructose uptake under steroid administration, and the transporter abundance as well as the mRNA for this same transporter were equally up-regulated, we could demonstrate transcriptional regulation of the functional activity of this transporters (for our example, GLUT5 would be the transporter). In fact one of your hypotheses consists of testing this possibilty according to our functional studies. Unfortunately, we were not able to demonstrate transcriptional regulation in any of the instances. Most of the findings supports posttranscriptional regulation, illustrated by dissociation among function, protein abundance and mRNA expression. This postranscriptional regualtion is supported by the literature when it regards SGLT1 [Hediger et al., 1987], but new when considering FABPs and GLUT5.

Among the possible signals involved in the phenotypic changes in sugar and lipid uptake, a great number of new genes have appeared in the recent literature [Bristol and

Williamson, 1988; Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Tappenden and McBurney, 1998; Reimer and McBurney, 1996; Jeppesen et al., 2001; Warner, 2001; Goyal et al., 1998; Rubin et al., 1998; Erwin et al., 2000; Stern et al., 2001; Fiochi, 1997; Kagnoff et al., 1996; Hardin et al., 2000], but the most studied ones that are strongly associated to intestinal adaptation are glucagon, ERG, ODC and cytokines. It is important to emphasize that the findings and variability of the different models of intestinal adaptation regarding mainly animal models, and time of the experiment are important aspects when considering analysis of this adaptive response. For instance, a signal or gene may be up-regulated soon after an experimental procedure, or the administration of a certain diet, but may be not changed or even may even be down-regulated days or weeks later. We choose to examine the effect of steroids over an intermediate time interval of four weeks, because in clinical practice steroids are given for several weeks. At this time frame we were not able to show the participation of c-jun, c-myc, c-fos, proglucagon, TNF-α, IL-2, IL-6 and IL-10, but we were able to show involvement of ODC in the prednisone effects on the ileal lipid uptake. Because of the time frame as discussed before, we appreciate that some of these signals could have been involved in the earlier intestinal response to steroids.

In summary, giving weanling rats four weeks of oral budesonide or prednisone in doses equivalent to those used in clinical practice (ileitis: 0.20 mg/kg/day; intestinal transplantation: 0.1 to 1.0 mg/kg/day; these experiments: 0.25 mg/kg/day) enhances the uptake of fructose and some lipids. These changes are not explained by variations in food intake or in the weight of the mucosa or in the villous height. The similar effects of a locally and a systemically acting steroid on fructose uptake suggests that the effect was produced at the level of the enterocyte, whereas the greater effect of PRED than BUD on lipid uptake raises the possibility that the mechanism was related more to some systemic effect. The alterations in nutrient uptake were not explained by changes in the abundance of transport proteins or their expression of their mRNAs, raising the possibility of post-translational modification.

2) Effects of Dietary Lipids

When adult rats are fed isocaloric diets varying in their type of lipids (SFA and PUFA), there is no difference in food intake or body weight gain [Thomson et al., 1986]. In contrast, when post weanling rats are fed these diets for four weeks, weight gain is approximately 30% higher in those fed PUFA as compared with SFA (Table 18). The explanation for the greater weight gain is not due to a difference in food intake, or to any alteration in the jejunal uptake of glucose (Tables 21 and 22) or fructose (Table 23). Indeed, ileal uptake of glucose was lower in PUFA than in SFA, and jejunal as well as ileal uptake of fructose was lower in PUFA than SFA. The differences in weight gain in PUFA versus SFA are also not explained by changes in lipid uptake (Table 38). In fact lipid uptake is higher in SFA than in PUFA (Table 38). These studies do not provide an explanation for the greater weight gain in rats fed PUFA than SFA, but the explanation likely relates to variations in metabolism because food intake was similar and absorption was lower, not higher. Although SFA stimulate lipid uptake, it is possible that these lipids are not as well delivered to the blood and lymphatic system and that the absorption of lipids *in vivo* would be less when rats are fed SFA than PUFA, even though the initial step was greater. It is important to remember that the uptake was measured *in vitro*, meaning that *in vivo* uptake might have been different from that represented in the experiments. Therefore, alterations of uptake *in vivo* might explain the alterations observed in the weight gain in animals fed SFA versus PUFA.

In adult rats, feeding SFA increases the value of the Vmax for glucose uptake in the jejunum and ileum [Thomson and Rajotte, 1983]. This was observed only for the ileum in these young animals (Table 21). Changing dietary lipids also had no effect on the passive uptake of D-mannitol or L-glucose (Table 22). The reason for the lack of change in glucose uptake in the jejunum in response to feeding SFA was not established in this study. We suggest that this is not due to a lack of response to dietary lipids, because the ileal uptake of glucose was greater in animals fed SFA as compared with PUFA (Table 21). SGLT1 protein abundance and mRNA expression did not correlate with uptake (Tables 26 and 28). Actually, in some instances, the abundance and expression of SGLT1 was reduced in SFA fed animals as compared to PUFA fed ones. The changes in glucose uptake were not the result of alterations in the Na^+ gradient predicted by any change in the abundance or expression of Na^+/K^+ ATPase (Tables 26 and 28). Therefore, the same possibilities discussed regarding the effect of steroids on fructose uptake and no effect on GLUT5 should be mentioned when analysing the effect of dietary lipids on glucose uptake: 1) phosphorylation/glycosylation; 2) recruitment along the villous-crypt unit and; 3) the role of GLUT2.

Fructose uptake is increased in weanling rats fed chow or SFA but not PUFA (Table 23). Differently from the effects of SFA on glucose uptake, fructose uptake was increased in both jejunum and ileum. Therefore, the mechanism by which dietary lipids modify glucose uptake is probably different than that related to fructose uptake and they may be site specific. More discussion related to the importance of the different segments of the gut will be presented when discussing intestinal resection. Once again, the same situation observed with steroids when affecting fructose uptake was observed with dietary lipids: changes in transport acitivity without correlation to the abundance and expression of the transporter when measured by Western and Northern blotting. Particularly in this model of intestinal adaptation (dietary lipids) we were able to perform immunohistochemistry and test the possibility of recruitment along the villous-crypt unit. We were able to realize an over expression of SGLT1 and GLUT5 in the situations where we had an enhancement of these transport activities. It is important to emphasise that although the total abundance was quantified and noted to be higher in the described groups, we did not observe the phenomenon of recruitment previously suggested in this discussion. This discrepancy between Western blotting and immunohistochemistry in terms of protein abundance was not clarified in this study. We accept the fact that immunohistochemistry is a qualitative assay rather than quantitative, but even when assessed only visually (without quantification) the differences are evident. One important aspect to consider is that we isolated both BBM and BLM and then performed Western blotting, but we did not look at the intracellular pool of the transporters that may be stained and detected by immunohistochemistry. Therefore, this intracellular pool of transporters may account for the differences observed and explain the discrepancy obtained with these two techniques to assess protein abundance.

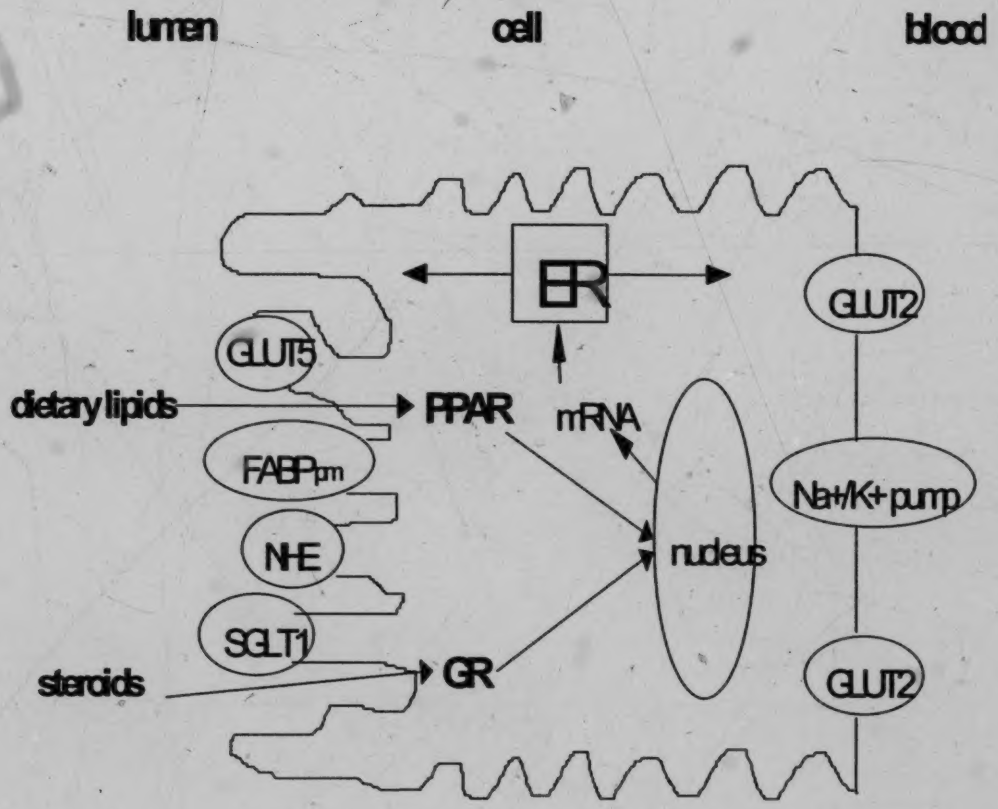
The uptake of lipids was increased by SFA in both sites jejunum and ileum, and this was not explained by increased expression of L-FABP or ILBP. Since we did not look at the protein expression of these two FABPs, and did not assess other FABPs such as I-FABP and FAT, we cannot discard the involvement of these proteins in this adaptive process. The signalling promoted by dietary lipid does not seem to involve ERG, ODC, IL-2, IL-6 or IL-10, but we were able to show in some instances the participation of proglucagon and TNF-α . This is a important finding that confirms the influence of dietary lipids on the intestinal gene expression. Therefore, we clearly present in this study effects of dietary lipids on the function of the small intestine as well as changes in gene expression. Although we could not directly correlate the findings in gene expression with the phenotypic alterations, we speculate that alterations in genes not analysed here or even the regulation of these genes will result in changes in the phenotype.

3) Interaction between steroids and dietary lipids

Steroids diffuse freely throughout the membranes because of their lipid soluble properties [Haynes and Larner, 1975]. Once in the cytoplasm, steroids bind to specific receptors termed GR [Haynes and Larner, 1975; Bamberg et al., 1996]. This receptor belongs to the family of supereceptors that includes PPAR and retinol binding protein [Alberts et al., 1994; Schulman et al., 1994; Sheppard et al., 1999]. The complex agonist plus the receptor undergoes structural activation that might involve heat shock proteins, dimerizes and it is now able to translocate to the nucleus where it interacts with specific DNA sequences termed GRE and influences gene transcrition[Chalepaski et al., 1990; Drouin et al., 1992]. The interaction of dietary lipids with the molecular mechanisms by which steroids alter gene expression becomes fascinating when Clarke and colleges, proposed that dietary lipids also cross membranes and bind to PPAR [Jump and Clarke, 1999]. Like steroids, this complex then migrates to the nucleous and binds to specific sequences and influence the machinery of transcription. One aspect that strongly suggests interaction of dietary lipids and steroids is the fact that PPAR also belongs to the GR family of receptors. Having to dimerize before being able to translocate, one could speculate that GR plus steroids and PPAR plus dietary lipids could migrate together to the nucleous instead of two GRs complexes or two PPARs complexes. This was not a hypothesis formulated at the beginning of our study, and for that reason no experiments were designed to prove this speculation.

The enhancing effect of steroids on fructose uptake observed in rats fed SFA can be prevented by feeding PUFA. The mechanisms involved in this inhibitory action might be associated to the same negative effect observed in resected animals fed PUFA when compared to those fed SFA [Keelan et al., 1996]. It would appear that there is a degree of specificity of the diet effect (fructose but not glucose uptake), but it is unclear why the diet effect may be different for the jejunum than for the ileum. The abundance of GLUT5 when assessed by immunohistochemistry was able to explain this enhancement in transport activity, but when the same measurements were analysed by Western blots, we were not able to detect any differences. The reason for this discrepancy has been explained previously.

Figure 13. Steroid-Lipid Interaction


lumen
cell
blood
ER
GLUT2
GLUT5
dietary lipids
PPAR
mRNA
FABPpm
Na+/K+ pump
NHE
nucleus
SGLT1
GR
steroids
GLUT2

The effect of BUD on lipid uptake depends upon the lipid composition of the diet: in animals fed PUFA, BUD increases the rate of jejunal uptake of the long-chain length fatty acids 16:0, 18:1 and 18:2, as well as the ileal uptake of 18:1 (Table 38). In animals fed SFA, giving BUD reduces the jejunal uptake of cholesterol and the ileal uptake of 16:0 (Table 38). By the same token, giving PRED to animals fed PUFA increases the jejunal uptake of 12:0, 18:1 and 18:2, and as well increases the ileal uptake of 18:3 (Table 38). However, giving PRED to rats fed SFA has no effect on lipid uptake in the jejunum, and reduces ileal uptake of 16:0 (Table 38). Thus, the ability of steroids to alter intestinal lipid uptake depends upon whether the animal is fed a diet enriched with PUFA or SFA. Feeding PUFA increases the effect of these steroids on the absorption of lipids.

We did not establish the mechanism by which a PUFA prevents the PRED- or BUD-associated enhanced uptake of fructose observed in rats fed a SFA, nor did we establish the mechanism by which PUFA increases the effect of those steroids on the absorption of lipids. However, we were able to discard ERGs, ODC, TNF-α, IL-2 and IL-6 in this response, and we were able to demonstrate the participation of proglucagon and IL-10 in some of this situations. For instance, the expression of proglucagon was increased in the ileum of animals given prednisone and budesonide and fed PUFA. PUFA prevented the effect of steroids on fructose uptake, and perhaps the mechanism involved in this suppression involves proglucagon or IL-10 signalling (Table 36). SFA reduced IL-10 expression in the gut as compared to PUFA in those animals given budesonide (Table 42). One could again speculate that the phenotypic alterations cause by dietary lipids could involve signalling by IL-10. Clearly, these locally and systemically active steroids, when given in doses which are effective clinically, modify the intestinal absorption of fructose and lipids by a process which is influenced by the dietary content of lipids.

4) Effect of Resection

We chose a protocol of non-massive intestinal resection (50% enterectomy) where the remaining proximal and distal intestinal remnants were adequate to assess the morphology and function at these sites [Keelan et al., 1996]. We also wanted to approach the clinical situation where a patient has Crohn's disease, has to have an extensive small intestinal resection and has to be on long-term use of steroids to suppress residual active disease.

When analysing the other studies in the literature, one needs to consider the extension and site of resection. For instance, an 80% enterectomy and dexamethasone (128 μg/kg, subcutaneously) which has been reported previously to blunt the expected increase in the bowel content of DNA one week after the procedure [Park et al., 1994], was not demonstrated when the same conditions were performed in a 50% resected model (Table 46). This lack of detrimental effect of dexamethasone may have also been due to differences in animal strain and their age, but the most significant differences were the length of intestine resected (50% versus 80%), the time after resection when our studies were performed (1 week versus 2 weeks), or the total dose of dexamethasone used (896 μg versus 1792 μg).

In our model, the animals' food intake, body weight gain, intestinal weight, and the percentage of the intestinal weight comprised of mucosa were unaffected by intestinal resection. Thus, the alterations in the function of the non-resected intestine in animals given steroids were not due to changes in these end-points.

With the removal of the middle half of the small intestine in this study, there was no change in the uptake of D-glucose (Table 47), L-glucose (Table 49), D-fructose (Table 51), 16:0, 18:2 and cholesterol (Table 65), as compared with transected animals. We could have subjected the animals to a massive small bowel resection and potentially been able to show enhanced uptake of sugars and lipids. However, the interpretation of the results would then have been more difficult, because of the expected concomitant changes in food intake, body weight gain and intestinal weight. Furthermore, we wished these studies to have some potential clinical relevance as mentioned before. For example, in patients with Crohn's disease, many of these individuals may have previously undergone an intestinal resection, but massive resections would be unusual.

The resection-associated alterations in the mRNA expression such as reduction in the jejunal expression of ODC and L-FABP, were not accompanied by variations in the uptake of lipids (Tables 63 and 67). We suggest that these alterations in gene expression were insignificant to lead to an adaptation of lipid and sugar uptake. It is unclear what is the role of the altered expression of these genes. The changes observed do no support the involvement of ODC in proliferative events at this particular time frame. Speculations point to up-regulation of ODC at some particular time that was missed in our study. L-FABP expression do not discard the involvement of this protein, since we did not assess the protein expression of this lipid binding protein. We cannot also discard the involvement of other fatty acid binding proteins such as FAT and I-FABP.

Although we were not able to demonstrate any significant changes after intestinal resection, we could use this model to demonstrate the interaction of the effects of steroids and dietary lipids with this adaptive model, as well as the adaptive response of the remaining intestine that even with half of this length keeps the absorptive capacity of a intact intestinal tract. The signals responsible for this adaptive response remain unknown but do not involve ODC, enterogucagon and ERG, at least at the time frame we performed our studies. These findings do not discard the possibility of these signals being up-regulated during the period we did not measure their expression (2 week-period). We could speculate that the ERG expression would be acutely stimulated (first 24 hours for instance), and would lead to a release and consequently overexpression of enteroglucagon (first 3 days). Once stimulated, the gut would respond by proliferative events that would involve ODC expression (first week). We did not perform these kind of experiments because reasons described before as well as because we were using an animal model. Cell cultures would definitely facilitate this sort of analysis.

5) Effect of Resection and Steroids

In resected animals, neither budesonide nor prednisone altered the weight of the intestine, nor the percentage of the intestinal wall comprised of mucosa (Table 46). It is possible that had we subjected the animals to a massive bowel resection (80% of small bowel removed), then the weight of the remaining intestine per unit length or surface area might have increased. Nonetheless, based on this data two weeks following a 50% enterectomy, it is clear that these steroids given in these doses and by these routes had no adverse effects on the weight of the intestine. Thus, the data suggest that the effects of steroids on the absorptive functions of the intestine, to be discussed, were not due to any steroid-induced change in the mass of the intestine.

Glucocorticosteroids are used to treat patients with a variety of intestinal conditions, including Crohn's disease and ulcerative colitis. Clinical studies have focussed on the use of the potent locally acting steroid, budesonide [Danielson et al., 1992; Matzen, 1991; Hanauer et al., 1995; Cortot et al., 2001; Levine et al., 2001; Tremaine et al., 2001]. Prednisone and budesonide modify the morphology and absorptive function of the intestine in young rats with an intact intestinal tract as discussed before. For instance, budesonide and prednisone increase fructose uptake (Table 9) . In this section we discuss the influence of the locally active budesonide versus two systemically active steroids (prednisone given by mouth, and dexamethasone given by subcutaneous injection) in adult animals in which a portion of the jejunum and ileum remained after removal of the middle half of the small intestine.

The doses of prednisone and budesonide used were chosen on the basis of regimens which have been shown to be clinically useful in humans [Greenberg et al., 1994; Rutgeerts et al, 1994]. The dose used has been shown to modify intestinal absorption of fructose and some lipids in chow-fed rats with an intact intestine as previously discussed. There is a difference in the potency of various steroids on the absorption of sugars after a 50% enterectomy: prednisone had no influence on the value of the Vmax of glucose uptake or on the uptake of fructose, whereas budesonide increased by over 120% the value of the Vmax for jejunal uptake of D-glucose (Tables 48 and 52). As well, budesonide increased by 70% the ileal uptake of fructose (Table 52). This occurred without any change in the non-mediated passive component of sugar uptake, as measured with L-glucose (Table 50). Thus, the locally active steroid budesonide accelerates the intestinal absorption of these two sugars following intestinal resection whereas prednisone and dexamethasone do not change the absorption of sugars in the small intestine. This might have a beneficial effect on those severely malnourished patients on steroids.

The protein abundance and the mRNA expression of the transporters responsible for the brush border membrane uptake of glucose and fructose (SGLT1 and GLUT5 respectively) did not correlate with the changes in the activity of these transporters. For instance, the enhanced jejunal glucose uptake with budesonide (Table 48) was not accompanied by an increase in the abundance of SGLT1 or its mRNA (Tables 55 and 56). Also, the enhanced fructose uptake with budesonide in the ileum (Table 52) was not accompanied by changes in GLUT5 abundance or mRNA expression (Tables 57 and 58). We did not perform immunohistochemistry of SGLT1 or GLUT5 because of the lack of adequate amounts of tissue (50% resection). It is possible that steroids may modify sugar uptake by changing the distribution of these transporters along the villus, thereby increasing uptake without any variation in the total abundance of transporters. We also could have observed changes in the abundance of SGLT1 and GLUT5 in animals given budesonide as compared with those given prednisone and dexamethasone when measured by immunohistochemistry, as we realized before when analysing the effect of steroids and dietary lipids on the absorption of sugars in animals with a intact intestinal tract The reasons why we were not able to demonstrate this overexpression of transporters by Western blotting has been discussed before. Also, Kellett and colleagues have provided evidence to suggest that under some circumstances when the activity of SGLT1 is stimulated, the increased cytosolic levels of PKC βII facilitate the trafficking of GLUT2 to the BBM, thereby providing and additional transporter for glucose and fructose [Kellet et al., 2001]. Thus, it is possible that a post-translational event with or without the involvement of another carrier is involved in the regulation of sugar uptake in response to the administration of steroids in resected animals.

Although the systemic bioavailability of budesonide is approximately one order of magnitude lower than for prednisone, its potency is much higher [Brattsand, 1990]. We speculate that the stimulating effect of budesonide on the jejunal uptake of glucose by SGLT1 and the ileal uptake of fructose by GLUT5 is the result of its greater effect on the enterocyte receptors for glucocorticosteroids. This adaptive response with budesonide following intestinal resection may be important to maintain the well-being of the animal. The glucose and fructose absorption-promoting effect of budesonide following intestinal resection may prove to be a useful agent to enhance the intestinal adaptive response.

The dose used in this study has been shown to modify intestinal absorption of some lipids in chow-fed rats with an intact intestine (Table 14). None of the three steroids affected the jejunal or ileal uptake of palmitic or linoleic acids or cholesterol in resected animals (Table 66). Adaptation of intestinal uptake of lipids is associated with changes in the intestinal brush border membrane (BBM) and enterocyte microsomal membrane phospholipids or phospholipid fatty acids [Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1986; Keelan et al., 1987; Keelan et al., 1989; Keelan et al., 1993], as well as phospholipid and fatty acid metabolizing enzymes [Garg et al., 1988; Garg et al., 1990]. The only exception to this general rule has been the lack of effect of variations in dietary lipids on the phospholipids in BBM, which under the same experimental conditions do demonstrate changes in lipid uptake [Keelan et al., 1987].

In this study we did not measure BBM lipids, so we are not able to comment upon whether the absence of an effect of steroids on lipid uptake was due to their lack of effect on BBM lipids or on the protein-mediated components of uptake [Schoeller et al., 1995a; Schoeller et al., 1995b; Poirier et al., 1996; Ibrahimi et al., 1996; Keelan et al., 1996]. There are lipid binding proteins in the intestine [Schoeller et al., 1995a; Schoeller et al., 1995b; Poirier et al., 1996; Niot et al., 1997; Storch 2001], but L-FABP is unlikely to play a major rule, as suggested by the marked decline in jejunal L-FABP mRNA (Table 39), but no alteration in lipid uptake (Table 38). Recent support for the uncoupling between intestinal lipid uptake and lipid binding proteins comes from the findings that 1) clones of mouse with disrupted genes for the intestinal fatty acid binding protein (I-FABP) are viable, have elevated plasma triacylglycerol and weigh more than the wild type [Vassielva et al., 2000]; and 2) dietary and diabetic alterations in lipid uptake are not paralleled by similar alterations in lipid binding protein expression [Drozodwski et al., 2001]. Our findings support this general concept. We add the cautiounary comment , however, that we did not measure the BBM protein content of L-FABP and ILBP, and there may as well be other lipid binding proteins which we did not measure. Immunohistochemistry of fatty acid binding proteins also could demonstrate alteration in the distribution of these proteins along the crypt villus unit, but that was not performed because of lack of tissue.

6) Effect of Resection, Steroids and Dietary Lipids

In this study, we have shown involvement of c-jun, proglucagon and ODC in the adaptive response. For instance, resected animals fed SFA and given budesonide increased their jejunal expression of c-jun as compared to those fed PUFA or chow and given budesonide (Table 91), steroids reduced the jejunal expression of c-jun in resected animals fed chow (Table 92), resected animals fed SFA and given budesonide had a reduction in jejunal ODC expression as compared to those fed chow or PUFA (Table 94), prednisone given to rats fed SFA increased their jejunal expression of proglucagon but steroids given to rats fed chow reduced the jejunal expression of proglucagon (Table 95), and intestinal resection reduced the jejunal expression of ODC (Table 97). It is not known the mechanisms by which these signals might alter the phenotypic alterations observed in transport activity. Of course, other signals may be involved in this adaptive response. Epimorphin/syntaxin 2 mRNA (early stages - 4 to 16 hours after 70%resection) that codes for a membrane-associated protein involved in morphogenesis of the lungs and skin, and PC4/TIS7 (early stages - 16 to 48 hours after 70% resection), a gene involved in nerve growth factor-mediated cytodifferentiation, may be among other signals that might be involved in the adaptive response after intestinal resection [Goyal et al., 1998; Rubin et al., 1998] . New signals involved in the adaptive intestinal response after resection have recently been identified by cDNA microarray analysis (3 days after 50% resection) such as the small proline-rich protein 2, involved in wound healing; glutathione reductase, a gene involved in intestinal apoptosis; NF-2 family members, also involved in apoptosis; etoposide-induced p53-mediated apoptosis; basic Kruppe-like factor, a transcription factor that activates the promoter for IGF; and prothymosin-α , involved in cell proliferation [Stern et al., 2001; Erwin et al., 2000].

Isocaloric changes in dietary lipids modify the intestinal absorption of nutrients in adult rats, with greater uptake of sugars, amino acids and fatty acids in animals fed a semisynthetic SFA versus PUFA diet [Keelan et al., 1996; Thomson et al., 1982]. This observation has been applied to purposely modify the adaptation of the intestine in a manner which achieves a therapeutic objective. For example, feeding SFA enhances uptake and prevents or reduces the malabsorption which otherwise would occur following the chronic intake of ethanol [Thomson et al., 1991], or following abdominal irradiation [Thomson et al., 1989]. Furthermore, the undesired enhanced uptake of sugars and lipids which occurs in experimental diabetes mellitus may be prevented by feeding PUFA [Thomson et al., 1987]. This has the additional clinical advantage of improving glucose control, and reducing the hyperlipidemia and elevated concentrations of hemoglobin A1c [Thomson et al., 1987; Thomson et al., 1988; Keelan et al., 1989]. Indeed, the desired enhancement of nutrient transport in rats following a 50% intestinal resection requires the presence of SFA in the diet.

Only in resected rats fed SFA was prednisone associated with a reduced absorption of fructose (Table 78). This malabsorption of sugars with prednisone was of potential nutritional significance, since it was associated with lower body weight gain (Table 70). Therefore, the adaptive response after a 50% intestinal resection may be damaged by the administration of systemic steroids such as prednisone, and this may have clinical manifestations such as weight loss. This intriguing malabsorption caused by prednisone in a resection model is probably due systemic manifestations of this drug since budesonide was not able to produce such effects.

Steroids may increase fructose uptake in animals with an intestinal resection, but this beneficial effect is prevented in rats fed SFA rather than PUFA. It is possible that in the setting of bowel resection, budesonide might be advantageous as compared with prednisone, with greater ileal uptake of fructose (Table 78), and with no unwanted loss of body weight (Table 70). It remains to be determined whether budesonide may play a therapeutic role to stimulate intestinal adaptation following intestinal resection in humans.

None of these three steroids affected the jejunal or ileal uptake of palmitic (16:0) or linoleic acids (18:2) or cholesterol in resected rats fed chow, SFA or PUFA (Table 97). The adaptation of intestinal uptake of lipids is associated with changes in the intestinal brush border membrane and enterocyte microsomal membrane phospholipids or phospholipid fatty acids [Garg et al., 1988; Garg et al., 1990; Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1986; Keelan et al., 1987; Keelan et al., 1993], as well as some phospholipid and fatty acid metabolizing enzymes [Keelan et al., 1985a; Keelan et al., 1985b; Keelan et al., 1985c; Keelan et al., 1986]. The only exception to this general rule has been the lack of effect of changes in dietary lipids on the phospholipids in the brush border membrane, which do demonstrate changes in lipid uptake under the same experimental conditions [Keelan et al., 1987]. In this study we did not measure brush border membrane lipids, so we are not able to comment upon whether the lack of effect of steroids on fatty acid uptake was due to their failure to affect the brush border membrane lipids or the protein-mediated component of uptake [Schoeller et al., 1995a; Schoeller et al., 1995b; Ibrahimi et al., 1996; Poirier et al., 1996]. Furthermore, steroids and dietary lipids did not modify the protein-mediated component of lipid uptake, as measured by the expression of the mRNAs for ILBP and L-FABP. The jejunal L-FABP mRNA was reduced in resected animals, but this did not contribute to any change in lipid uptake.

Recent support for a dissociation between intestinal lipid uptake and the abundance of lipid binding proteins comes from the observation that clones of mice with disrupted genes for the intestinal fatty acid binding protein (I-FABP) are viable, have elevated plasma triacylglycerol, and weigh more than do the wild variant [Vassielva et al., 2000]. It is appreciated that the study illustrates the effects of another lipid binding protein, I-FABP, which was not analyzed in this study. It is also important to remember that we looked at the lipid binding protein mRNA expression, and it is possible that alterations in the protein abundance could have been observed. In order to discard the involvement of lipid binding proteins in the response observed in resected animals, further studies assessing protein abundance and expression of other lipid binding proteins must be performed.

7) Conclusion

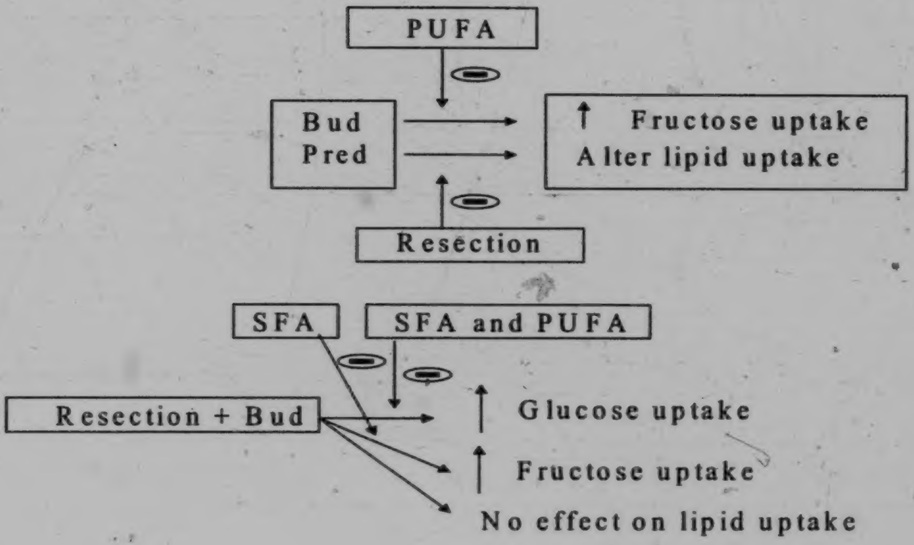
The first conclusive aspect and the most obvious one is that steroids and dietary lipids interact and act differently when looking at the function of the gut. For instance both prednisone and budesonide increase the uptake of fructose but this can be prevented by feeding a polyunsaturated fatty acid diet. This interaction is very significant when considering that according to the diet, steroids might differently affect the function of the gut. It is important to remember that we did not assess other functional parameters (such as pulmonary function and absorption of amino acids) that might also be changed or influenced by steroids and dietary lipids.

The adaptive response due to intestinal resection blocks the effect glucocorticosteroids and dietary lipids on the intestinal absorption of lipids. The mechanisms are unclear, but if the effect of dietary lipids and the steroids involve for instance PPAR and GR, resection signals block the translocation of these receptors to the nucleous.

Unfortunately we were not able to discover the signalling cascade involved in the adaptive response to steroids, dietary lipids and intestinal resection. However, at least in some instances we showed the involvement of proglucagon, ODC and c-jun. Furthermore, we observed the complexity of this adaptive process and no doubt that future design studies based in this adaptive model will be created.

Budesonide significantly increased the absorption of sugars in resected animals as compared to the other two systemically acting steroids. If this is true for humans, important contributions for patients nutritionally compromised, such as the ones with inflammatory bowel disease, could be obtained with the use of budesonide instead of prednisone.

Figure 14. Summary of Results


PUFA
Bud
Pred
↑ Fructose uptake
Alter lipid uptake
Resection
SFA
SFA and PUFA
Resection + Bud
↑ Glucose uptake
↑ Fructose uptake
No effect on lipid uptake

8) Future Studies

In relation to the signalling responsible for the adaptive response to steroids, dietary lipids and intestinal resection, time course studies would provide important information regarding how the signals are regulated in different time points. We speculate that ERG would be up-regulated during the first week of administration of steroids, dietary lipids and intestinal resection. Even hours or days after the treatments or procedures could be assessed. All the other signals studied such as ODC, proglucagon and cytokines could be up- or down-regulated at specific time. It would be also of value, to assess the mRNA expression of nutrient transporters such as SGLT1 and GLUT5 in a time line manner as described for the signals, in the specific models were glucose or fructose were up-regulated. For instance, in animals given budesonide, fed chow and undergone intestinal resection. Information regarding regulation of these transporters would be provided by such experiments.

cDNA array technology would have given us significant information regarding the specific genes that were up- or down-regulated at the time frame assessed. However, the technique is still very expensive and we would no hesitate to use it if enough mRNA were available to perform such a technique.

The well described interaction between dietary lipids and steroids requires further investigation, specially regarding the expression of PPAR and SR.

Immunohistochemistry was of significant value when used to explain the phenotypic findings in gut function. The explanation for the changes in the uptake of nutrients might lay on immunohistochemical sections that we were not able to perform in some models for lack of tissue.

One of the possible explanations for the dissociation between uptake and protein abundance could be clarified by the use of 2-dimensional gel electrophoresis which would allow us to analyse small differences in the molecular weight of the same proteins such as SGLT1, suggesting phosphorylation and/or glycosylation of these transporters. Perhaps SGLT1 has to be phosphorylated and/or glycosylated in order to be able to reach the BBM and work. Other possibility of assessing these two pools of SGLT1 or even the other transporters would be a combination of electron microscopy and immunohistochemistry.

In terms of clinical possibilities, it would be very intriguing to observe in patients the nutritional status of patients when on budesonide or prednisone, specially on those undergone intestinal resection due to short bowel syndrome. Accordingly to our findings possible significant changes could be found. The same could be said for the diets, but we understand the difficulties presented when analysing and controlling dietary factors.

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P) **APPENDIX I**: Immunohistochemistry for GLUT 5

Control

Budesonide

Prednisone